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ON THE TOXIC EFFECT OF DELETERIOUS AGENTS ON THE GERMINATION AND DEVELOPMENT OF CERTAIN FILAMENTOUS FUNGI.[1]

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INTRODUCTORY.

THE object of the writer in undertaking the investigation, the results of which are presented in this paper, was to determine approximately the relative and absolute toxic properties of a few deleterious agents as shown by their influence on the spores, the mycelium, and the fructification of certain of the mold fungi.

In recent years the study of plant pathology has come to be one of the most important in the whole range of botanical research. It seems desirable, therefore, that all possible light should be thrown upon the toxic properties of the various agents used in combating fungus pests. It is also very desirable, from a scientific point of view, to throw all light possible upon the problem as to the *element* or *group of elements* to the influence of which the toxic properties of the compound are to be attributed.

Thanks to the progress of modern physical chemistry, plant physiologists are now enabled to make up solutions of all chemi-

[1] A thesis presented to the Faculty of Cornell University for the degree Master of Arts.

cal agents having the same number of molecules present in equal volumes of the various solutions. This is a decided step in advance, inasmuch as it enables investigators to compare the properties of a molecule of any substance with those of any other molecule, a comparison obviously impossible under the old method of making up solutions of a certain per cent. by weight. The vital error in making a comparison between percentage solutions is readily seen when we recall that a 1 per cent. solution of formaldehyde contains over eight times as many molecules per cc. as a 1 per cent. solution of mercuric chloride.

These equi-molecular solutions are made by dissolving as many grams of the compound as there are units in its molecular weight in 1000 grams of the solvent. Such a solution is termed a "*normal solution*" of that compound, and it is represented conveniently by the formula $\frac{n}{1}$. Diluted to half this strength we get $\frac{n}{2}$, or one half normal solution, and so on. Solutions stronger than normal may be made by using double or quadruple the number of grams specified to the 1000^{cc} of the solvent, giving $\frac{2n}{1}$ and $\frac{4n}{1}$, respectively, of the substance.

Still more recent chemical and physical research has shown that in the case of very many substances in solution, many of the molecules of the dissolved substance are no longer present as such, but have become divided into two or more parts. These part-molecules have been termed *ions*. To illustrate, let us suppose that 36.37 grams of pure HCl have been added to 1000 cc. of water; we know that the HCl is no longer all present as whole molecules. It fact, we have every reason to believe that about 80 per cent. of it has become *ionized* into $\overset{+}{H}$ and $\overline{Cl}$ ions, the $\overset{+}{H}$ ions being charged with positive electricity, the $\overline{Cl}$ ions having a corresponding negative charge. The ions of all substances capable of ionization are similarly charged, and have been named *cathions* and *anions*, respectively, from the fact that those charged with positive electricity (cathions) migrate

towards the cathode when a current of electricity is passed through the solution, while those charged with negative electricity migrate in the opposite direction towards the anode. As we have already said, a $\frac{n}{1}$ solution of HCl is about 80 per cent. ionized. This percentage of ionization increases with each increase of dilution, becoming practically complete at $\frac{n}{1000}$ in the case of HCl. Limited space forbids further discussion of this interesting and important phenomenon. For further information the reader is referred to the excellent papers by Drs. Kahlenberg and True ('96) and Mr. F. D. Heald ('96), or to the more recent text-books on physical chemistry. Suffice to say in this place that the ionization of the molecule has enabled chemists and physiologists to determine in many cases the *toxic element* or *group* in poisonous compounds. We shall have occasion to refer frequently to this matter in discussing the experimental data presented in this paper.

It has been the writer's aim to supplement the work of Drs. Kahlenberg and True, and Heald on phanerogams, and Drs. Krönig and Paul ('97) on bacteria, by applying the theory of the ionization of the molecule to the study of the physiology of fungicides.

METHODS.

Selection of forms.—In the selection of forms the following points were given particular consideration: (*a*) *regularity of germination*, (*b*) ability to grow normally in liquid media, (*c*) ability to fruit normally in a saturated atmosphere. After experimentation with a large number of forms the following were chosen as being well suited for the work: *Aspergillus flavus* (?) Link, *Sterigmatocystis nigra* v. Tieghem, *Œdocephalum albidum* Saccardo, and *Penicillium glaucum* Link. *Botrytis vulgaris* Fr. was afterwards chosen as a fifth form, it being entirely satisfactory in regard to germination and mycelial development, and especially desirable because of its semi-parasitic habit; it, however, failed to fruit in cell cultures. Pure cultures of these

molds were obtained and renewed from week to week. The spores used in inoculating the cultures in the experimental work were taken from fresh tubes in which the fungus had been growing seven to fourteen days. A solid medium made by adding 12 gm of agar to a liter of sugar beet infusion was found to be very satisfactory for stock cultures.

Selection of medium.—The object being to test the effect of the deleterious agents on the fungi under as nearly normal conditions of development as possible, the selection of a suitable medium was of primary importance. Many preliminary cultures were made with various media, including distilled water, infusions of potato, celery, sugar beet, prune, and bean (stems, pods, and mature seeds), besides various others compounded from inorganic salts, sugars, asparagin, etc. The results in germination and development were very varied. In distilled water Sterigmatocystis and Penicillium failed to germinate in 24 hours at 28° C., and of Botrytis, which did the best of the five forms, but 40 per cent. germinated in that time. Mycelial development in all was meager, and fruiting generally *nil*. Very minute quantities of the deleterious agents were found to inhibit germination, but the *death-point* of the spore was found in the case of dichloracetic acid, potassium hydroxid, and cobaltous sulfate, to be the same as in the medium finally selected. The media compounded from salts, sugars, etc., were more satisfactory; the vegetable infusions, however, were superior to all others. An infusion of sugar beet was ultimately chosen as being on the whole the medium best suited for the forms used. The fact that, in the case of the three typical poisons mentioned above, the concentration causing the death of the spore proved to be the same, whether the agent was dissolved in distilled water or the beet infusion, is very important in that it shows that this medium, which was used throughout the study, does not perceptibly change the toxic properties of these agents towards the fungus spores. The infusion of sugar beet was prepared by steeping 450 grams of the root in a liter of water for 3 hours at 100°C. It was then strained, cooled, and stirred up with the whites of two

eggs, after which it was boiled, strained, filtered, and poured into flasks. After thorough sterilization the infusion was ready for use. In order to get the greatest possible uniformity it was found desirable to make up four or five liters at a time. In this medium the spores of all the forms used germinated quite uniformly in from 3 to 8 hours (according to the species), grew rapidly, and fruited normally (except Botrytis) in from 18 to 44 hours at a temperature of 28°C.

Method of culture.—The van Tieghem hanging-drop culture was found to be entirely satisfactory. The cylinder part, which was of glass, had an internal diameter of 17.5^{mm}, and a height of 10.7^{mm}. This cell, with a capacity of over 2.5 cubic centimeters, provided an abundance of oxygen for the normal development of the fungi. The cylinders were cemented to the slip by means of beeswax, and the cell was completed by sealing the cover to the top of the cylinder by means of a ring of vaseline, applied by inverting it on a glass slip covered by a thin layer of melted vaseline. A small nick was made in this ring so that when the cover was applied a minute opening might be left through which the expanding air could pass when the cultures were placed in the thermostat. An hour later they were carefully examined, and such as had not already become hermetically sealed were now made so by tapping with a pencil over the tiny opening referred to above. This precaution prevents much trouble and loss when culturing with volatile substances. For convenience in handling and examining under the microscope two cells were placed on each slip. The cells were permanently labeled by gumming lettered and numbered labels to the ends of the slips. As a precaution against accidents duplicate cultures were not placed on the same slip.

The various dilutions in beet infusion of the compound to be tested were made up in a dozen or more bottles of about 30^{cc} capacity. Each bottle was provided with a glass rod, drawn to a blunt point, by means of which the culture drop was transferred to the cover. *Four or five drops of the same solution were then placed in the bottom of the cell.* The spores of the fungus to

be tested were transferred from a pure culture to the hanging-drop by means of a sterile platinum needle, the utmost care being taken to *prevent the adherence of the spores in bunches* in making the inoculation. The cover bearing the culture was then inverted on the cell and gently pressed until completely closed, except for the minute opening already fully described. When a set of cultures was complete all were placed in a thermostat which was kept at a constant temperature of 28°C.

Care of cells, covers, pipettes, etc.—After completion of a series of cultures, all bottles, rods, etc., were thoroughly washed and placed in running water for several hours, then dried and placed in a dry oven at 160°C. When the cultures in the cells matured, the covers were removed and the cells were thoroughly washed under the water tap, wiped, air-dried, and finally placed in the dry oven at 110°–120°C. for an hour. This insured thorough sterilization, and at the same time drove off the last trace of any volatile substance that might have escaped the washing. The covers were first boiled in strong KOH, then in several changes of water; this was followed by boiling in strong $H_2SO_4 + K_2Cr_2O_7$. They were again thoroughly rinsed and again boiled in four changes of clear water, rinsed in 95 per cent. alcohol, wiped, and sterilized at 160°C. The pipettes were cleaned by forcing water through them for an hour by attaching to the water tap. They were then sterilized in a steam sterilizer.

An occasional culture was found to be contaminated with bacteria, due no doubt to dust particles bearing spores coming in contact with the cultures in the making. Such contamination, however, by bacteria or fungi amounted to less than 1 per cent. of the cultures made after the completion of preliminary experimentation.

Vapor pressures in the cell.—It has long been known to physical chemists that every liquid has a certain vapor pressure. Pure water at standard atmospheric pressure and 28°C., has a vapor pressure of 28^{mm} of mercury. Any addition of a substance or substances to this water will lower its vapor pressure. If the substance added be hygroscopic the lowering of the vapor

pressure may be very great, as is the case with KOH and H_2SO_4. On the other hand, should the substance be quite volatile the increased vapor pressure of the substance added may more than counterbalance the lowering of the vapor pressure of the water, as is the case when ammonia or alcohol is mixed with water. To recapitulate in brief, the addition of any substance or substances to water gives a mixture with a vapor pressure at variance with that of pure water. This vapor pressure may be greater or less than that of pure water, depending on the physical properties of the substance or substances added. Thus, a hanging-drop containing KOH in a cell in which water has been placed below (as is the usual method), absorbs moisture by reason of its low vapor pressure. Indeed, there will be a constant distillation of water vapor from the water below to the culture drop, until all the water has passed up or the drop, becoming too large to "hang," falls to the bottom of the cell. With alcohol the reverse takes place. No sooner is such a culture made up than the alcohol begins to distill from the hanging-drop, and it has not, even for an hour, the concentration supposed to be present. Such a culture as a test of toxicity is valueless.

Were it possible to have all hanging-drops of exactly the same size, and exactly the same quantity of water below, we should expect uniformity in results. Such uniformity, however, would be useless, perhaps worse than useless, as it might prevent attention being called to the fundamental error of the method, viz., the use of solutions of varying vapor pressures in the same cell. As a matter of practical experience, however, it is impossible to have all hanging-drops of exactly the same size, and erratic germinations are consequently the inevitable result. To quote from one of the more recent studies, Stevens ('98) found that of four cultures of Macrosporium in a $\frac{n}{819200}$ solution of mercuric chlorid, three grew and one failed; of eight cultures in $\frac{n}{409600}$ five grew and three failed; of ten cultures in $\frac{n}{204800}$ four grew and six failed; of four cultures in

$\frac{n}{102400}$ one grew and three failed! With a strictly non-volatile substance we should expect less variation, because with less difference between the vapor pressures of the hanging-drop and that of the water below, the changes in the concentration of the deleterious agent in the hanging-drop would necessarily take place more slowly. Of the volatile properties of $HgCl_2$ we shall speak later.

In looking for a method that would meet every requirement of the case, many preliminary tests were made with all five molds and with different deleterious agents. Two such tests with potassium cyanid are here presented in detail. It is of interest to note that while KCN is not in itself a volatile compound, in aqueous solution more or less hydrolysis takes place, resulting in the formation of a corresponding amount of HCN (Shields '93), which is quite volatile. Hence, aqueous solutions of KCN are in their behavior quite typical of volatile compounds.

Column 1 gives the culture label by means of which the cultures were identified; column 2 the concentrations of KCN in the hanging-drops of the various cultures; column 3 the solution—if any—used in the bottom of the cell. "Dry" implies that no water or other solution was placed in the bottom of the cell. Under the head "germination" the percentage of spores germinated is given for three observations, at 12, 24, and 36 hours, respectively. Under "development" the length of the germ tubes of spores showing an average development is given in micromillimeters.

These data not only show that cultures having *water* in the bottoms of the cells are unreliable and vary according to the *amount* of water present, but that "dry" cells are equally unsatisfactory. Especial attention is called to the record of the cultures labeled "A10." In these the culture drop contains but one fourth of the KCN present in cultures labeled "A9," the former, however, had several drops of the solution from which the hanging-drop was made placed in the bottom of the cell instead of the customary drop of water. The striking difference in the results

ASPERGILLUS IN BEET INFUSION + KCN

Temperature 28° C.

Culture label	Str'ngth of solution	Conditions of cell	Germination			Development			Notes
			12 hrs.	24 hrs.	36 hrs.	12 hrs.	24 hrs.	36 hrs.	
			%	%	%	μ	μ	μ	
A1	- KCN	(Check culture)	100			250	∞	∞	Fruited in 20 hrs.
A7	$\frac{n}{32}$	Dry	25	100		barely germ.	45	200	
A7	$\frac{n}{32}$	"	10	100		"	40	100	
A8	$\frac{n}{32}$	One drop of water	7	100		"	120	500	
A8	$\frac{n}{32}$	" "	5	100		"	100	800	
A9	$\frac{n}{32}$	Cell half filled with water	45	100		140	∞	∞	Well fruited at 38 hrs.
A9	$\frac{n}{32}$	" " " "	70	100		150	∞	∞	" "
A10	$\frac{n}{128}$	$\frac{n}{128}$ KCN solution	0	0	75	..	..	100	
A10	$\frac{n}{128}$	" "	0	0	10	..	..	80	

PENICILLIUM IN BEET INFUSION + KCN

Temperature 28° C.

Culture label	Str'ngth of solution	Conditions of cells	Germination			Development			Notes
			12 hrs.	24 hrs.	36 hrs.	12 hrs.	24 hrs.	36 hrs.	
			%	%	%	μ	μ	μ	
E1	−KCN	(Check culture)	100			110	∞		Fruited at 24 hrs.
E7	$\frac{n}{32}$	Dry	0	5	10	...	barely germ.		No development
E7	$\frac{n}{32}$	"	0	3	10	...	"	70	
E8	$\frac{n}{32}$	One drop of water	.1	10	25	barely germ.	20	150	
E8	$\frac{n}{32}$	" "	.01	10	20	"	20	130	
E9	$\frac{n}{32}$	Cell half filled with water	2	70		8	80	∞	Fruited at 36 hrs.
E9	$\frac{n}{32}$	" " " "	10	75		15	100	∞	" "
E10	$\frac{n}{64}$	$\frac{n}{64}$ KCN solution	0	0	0	...	...	...	
E10	$\frac{n}{64}$	" "	0	0	0	...	...	..	

needs no further comment. The results with Penicillium and other forms abundantly confirmed the test, and lead to the conclusion that *when water is used at the bottom of cells containing hanging-drop cultures and solutions of substances having very high vapor pressures are being tested, erratic germinations are to be expected, and in the case of a test of any substance—particularly those of high or low vapor pressure—the toxic properties shown will be less than a correct method would indicate.*

In the regular work reported in this paper, a few drops of the same solution as that used in the hanging-drop of any particular cell was in every case placed in the bottom of that cell, thus establishing complete equilibrium of vapor pressures in the cells, and thereby preventing changes in the concentration of the solutions under test as must take place when this precaution is not observed. By this method it was found quite feasible to make hanging-drop cultures of any composition whatever, ranging from a $\frac{16n}{1}$ (70 per cent.) solution of alcohol to a $\frac{4n}{1}$ (21 per cent. solution of potassium hydroxid.

Stock solutions of chemicals.—These were prepared in all cases by a responsible chemist from the purest chemicals obtainable. It was found desirable to have stock solutions in highly concentrated form so that all necessary diluting could be made by adding beet infusion. The stock solution of HCl, for example, contained 25 grams pure HCl per 100^{cc}. $\frac{n}{1}$ HCl contains 3.58 per cent. HCl by weight. Such a solution was gotten by taking 5^{cc} of the stock solution and diluting, by adding beet infusion, to 34.9^{cc}. $\frac{n}{2}$ was gotten by diluting 10^{cc} $\frac{n}{1}$ to 20^{cc}, and so on. Chemical agents liable to deteriorate in quality (*e. g.*, KCN) were titrated by the chemist on the morning of the day on which cultures with it were made up.

Trial and regular cultures.—Inasmuch as the work was in most cases of a pioneer character, it was found desirable to make up a large number of trial cultures preliminary to the regular work in order to get some definite idea as to the toxicity of the

various chemicals to this group of plants; for it was of course early learned that the data worked out for phanerogams by Kahlenberg and True ('96) and Heald ('96) were a contrast rather than a comparison when placed beside the facts learned by experiment from the fungi. Heald ('96) called attention to this fact on finding a fungus growing vigorously in a solution of HCl that had killed the root of Pisum. The data obtained from the trial cultures proved invaluable in making up the regular cultures, as it was then possible in most cases to make up a series of twelve dilutions in no. 1 of which the spores would certainly be killed, while in no. 12 the fungus would be practically unharmed.

In the regular work all cultures were made up in duplicate, including duplicate checks in pure beet infusion. Important points were quite frequently checked over in duplicate and sometimes in quadruplicate. This was also done in the case of *unexpected* developments; for instance, the writer was surprised to find the spores of Penicillium showing so great a specific resistance to acetic acid. Repeated checking, however, proved the correctness of the first observation.

One of the most marked features of the entire work was the *regularity in results*. It is true that the cultures nearest the inhibiting point in a few cases varied to the extent that germination of a number of spores took place in one culture while the duplicate failed. Such were not considered *erratic*. If, however, both cultures of a certain strength grew and one of some weaker concentration failed, the latter would be considered erratic. Such erratic cultures, however, did not exceed a dozen in a work requiring upwards of forty-five hundred regular cultures aside from preliminary work. Such as did occur were doubtless due to oversight in cleaning the cells or other accident.

Examination of cultures, noting results, etc.—Cultures were made up in the early part of the day, and were examined at intervals of from three to six hours for the fourteen hours following, and at longer intervals until the fungus had matured, or the spores in the cultures which had failed to germinate were transferred to pure beet infusion to test their vitality. The percentage of

spores germinated in the various cultures was noted on two occasions, first when from 30 per cent. to 70 per cent. of those in the checks had germinated, and again a few hours later. A similar method was adopted to indicate the early mycelial development. At some time after germination the germ tubes showing an average development were measured and noted; this was repeated a few hours later, and sometimes a third measurement was made. The first appearance of conidia was also noted, but as this very frequently occurred at night (between 15 and 22 hours after inoculation) the point was not so well noted.

In the earlier work, the cultures in which the spores *failed to germinate* were opened at 72 hours and a number of the spores were transferred by means of a sterile platinum needle to a hanging-drop of pure beet infusion in a clean cell, in order to test whether they were killed by the agent or merely inhibited. If 1 per cent. or more survived, the culture was classed as *inhibited;* if none at all or less than 1 per cent. survived, they were classed as *killed.* In most of the work, however, the transfers were made at 48 hours, it being found that all spores that could germinate did so in less than 36 hours and usually much less.[1]

The cultures which germinated in the presence of the deleterious agent were likewise divided into two classes: (1) those which although they may have been retarded or stimulated in mycelial development by the agent, finally matured a fair crop of conidia in about the normal time; (2) those, which, although they germinated and continued to grow, presented a markedly irregular or retarded mycelial development, and generally failed to fruit. Between these two classes came—as might be supposed—a number of cultures which were very difficult to classify. In some cases there would be an apparently normal mycelial development but almost total supression of fruiting. In other cases an irregular, meager, and even yeast-like mycelium would cause surprise by finally developing a number of apparently

[1] Cultures in alcohol, formaldehyde, H_2O_2, and KCN were transferred at 72 hours, all others at 48 hours.

normal fruits. In general, a fungus was said to be "injured" by that concentration of the deleterious agent which prevented its classification in class I as above.

In discriminating between class III (inhibited spores) and class IV (killed spores) attention is again called to the importance of avoiding *bunches* of spores in making the inoculation. In many solutions such bunches — doubtless containing air — float on the surface of the drop and fail to receive the full influence of the agent. When spores from such a culture are transferred to pure beet infusion to test their vitality the bunch may be broken up in the process and the spores germinate readily while all others are dead. Much can be done with care in making the inoculation, but at best it is a serious source of error, and it has been on this account, and on account of the impossibility of transferring the spores without taking with them small quantities of the agent, that the *inhibiting concentration* rather than that causing death has been adopted as the *chief critical point* in discussing the experimental data.

To illustrate what has been said regarding the taking of notes on cultures, a typical left-hand page of the culture notebook is here reproduced. This will also aid the reader to understand the classification of the experimental data presented later. The opposite page was always reserved for more extended notes on points observed from time to time in the progress of the experiment.

Sources of error.—Before proceeding to a discussion of the experimental data it might be well to mention briefly the sources of error observed during the preliminary study and guarded against in the progress of the work.

1. Xylonite cells were found to have an injurious influence on some fungi when used for hanging-drop cultures at 28°C.

2. Bacterial contamination.

3. Lack of equilibrium in vapor pressures in the cells.

4. Deterioration of stock solutions (see details of experiment with KCN).

5. Use of impure vaseline for sealing cells.

ASPERGILLUS IN BEET INFUSION + $CoSO_4$.

Cultured at 9 a. m., Feb. 21, 1899. Temperature. 28° C.

Chart	No. of culture	Culture label	Strength of solution	Germination				Mycelial development						Fruit hrs.	Notes
				hrs.	%	hrs.	%	hrs.	μ	hrs.	μ	hrs.	μ		
I.	2479	A1	$-CoSO_4$	6	85			6	30	12	200	22	∞	22	Record of duplicates omitted to save space
	2481	A2	$\frac{n}{2048}$	6	80			6	30	12	210	22	∞	22	
	2483	A3	$\frac{n}{1024}$	6	85			6	30	12	200	22	∞	22	
	2485	A4	$\frac{n}{512}$	6	80			6	30	12	180	22	∞	27	
	2487	A5	$\frac{n}{256}$	6	75	8	100	6	25	12	170	22	∞	27	
II. Intermediate	2489	A6	$\frac{n}{128}$	8	50	12	100			12	60	22	∞	36	
	2491	A7	$\frac{n}{64}$	12	1	22	100					22	170	48	Well fruited at 72 hrs.
	2493	A8	$\frac{n}{32}$	22	1	36	2					22	15	72	Few fruits only
III.	2495	A9	$\frac{n}{16}$	[Transferred at 48 hrs. 12 hrs. 5% germ.; 24 hrs. many more.]											
	2497	A10	$\frac{n}{8}$	[Transferred at 48 hrs. Failed.]											
Class IV.	2499	A11	$\frac{n}{4}$	"	"	"	"	"							
	2501	A12	$\frac{n}{2}$	"	"	"	"	"							

6. Use of spores of uncertain age and vitality.

7. Use of culture media unsuited for the normal development of the fungi tested.

8. Imperfect sealing of the cells, due generally to the raising of the cover by expansion of contained air when the cultures were placed in the thermostat.

9. Transference of bunches of spores in making inoculations.

EXPERIMENTAL DATA AND DISCUSSION.

Details regarding the critical points (*i. e.*, concentrations causing injury, inhibition, and death) of the various fungi in the more important of the thirty-seven compounds tested may be found by referring to the diagrams prepared to accompany this paper. This device, primarily intended to conserve space by giving in a condensed form the various specific resistances of the different molds will also be useful, it is hoped, in conveying to the reader, by means of the eye, a general impression as to the relative toxic properties of the agents tested. It should be distinctly borne in mind in consulting these diagrams that each vertical line represents a *doubling* of the number of molecules present in the solutions in passing toward the right, the whole space between two vertical lines representing one concentration. The relative *average* toxic properties of the more poisonous agents are further graphically depicted by means of the two charts in the final installment of this paper.

ACIDS.

In the case of acids, diagrams have been prepared, giving the resistances of the individual molds in the eight acids. These diagrams will be found to accompany the diagrams for the various acids on pages 307–308. In these diagrams an attempt has been made to emphasize the fact that the solutions double in concentration in passing to the right, by placing at the head of the columns the proportions of molecules present in each solution in terms of x; x being in every case the number of molecules present in a $\frac{n}{262144}$ solution. Thus a normal solution

always contains $262144x$ molecules, $\frac{2n}{1}$ contains $524288x$ molecules, and so on.

Hydrochloric acid, HCl; 70, *230*, 614. This acid, on account of its very high ionization at the critical points, and because of its very wide use in physiological and chemical investigations by other workers, has been taken as a *standard* by which we shall compare the others. In making these comparisons, HCl, being the most highly ionized of all acids, is *assumed* to represent the value of ionic $\overset{+}{H}$. This is a purely arbitrary assumption, and the reader will bear in mind that when we speak of "the toxic value of ionic $\overset{+}{H}$" we mean the nearest approach we could make to determining its toxic value for the molds, viz., *that of 91% ionic* $\overset{+}{H}$ *+9% HCl.*

The following "coefficients" have been worked out for HCl with these five molds: coefficient of injury, 70; coefficient of inhibition, *230;* coefficient of death-point, 614.

These coefficients mean that *on an average for the five forms used* $\frac{70}{2048}$ of a normal solution caused distinct injury to the cultures, $\frac{230}{2048}$ of a normal solution inhibited the germination of the spores, and $\frac{614}{2048}$ of a normal solution killed the spores. The denominator of the fractions, 2048—the eleventh power of 2—has been used throughout this paper in determining coefficients. As a matter of convenience the numerator only has been expressed in discussing the three critical points of the various agents, giving as it does at a glance the correct *relative* toxicity of the agents. The *absolute* value of any coefficient may (in either mold or agent) be determined by simply supplying the omitted denominator, 2048, the resulting fraction being in every case that proportion of a normal solution. In discussing the various agents these coefficients will be placed immediately after the chemical formulæ in the order given. That expressing the inhibiting value is *italicized* to emphasize the fact that it is regarded as the most significant.

From time to time attention will be called to the wonderful resistance of the fungi to many deleterious agents as compared with that of the higher plants. The comparison (or contrast?) in resistance to ionic $\overset{+}{H}$ is as 800 : 1, the average death-point for the five species of molds being $\frac{n}{3.3}$ HCl, while that of three species of phanerogams is $\frac{n}{2740}$ HCl. (Heald, '96, p. 152.)

On the whole, HCl was at the same time the most completely ionized and the least toxic of the acids tested. Sterigmatocystis proved most resistant, for although germination and early mycelial development were distinctly retarded by $\frac{n}{1024}$, $\frac{n}{4}$ was required to inhibit all the spores, and $\frac{n}{2}$ for forty-eight hours to kill them. Cultures in $\frac{n}{16}$, although much retarded in early development, had at forty-eight hours quite overtaken the checks, and at seventy-two hours far surpassed them in amount of mycelium produced. Distinct retardation of fruiting was first noticed in $\frac{n}{128}$; $\frac{n}{16}$ required nearly double the time to mature its fruit as

EXPLANATION OF DIAGRAMS.

The initials A., S., Œ., B., P. stand for the respective generic names of the fungi used.

The fraction of a normal solution placed at the head of a column refers to the *space between the vertical lines* over which it stands. In this space is depicted the result of culturing the various fungi in this concentration of the different agents by the symbols described below:

Two lines indicate normal or almost normal development.

Three lines indicate distinct injury.

Four lines indicate very great injury.

Alternate blocks indicate total inhibition of germination.

Solid black indicates death of spore when tested for vitality at 48 hours.

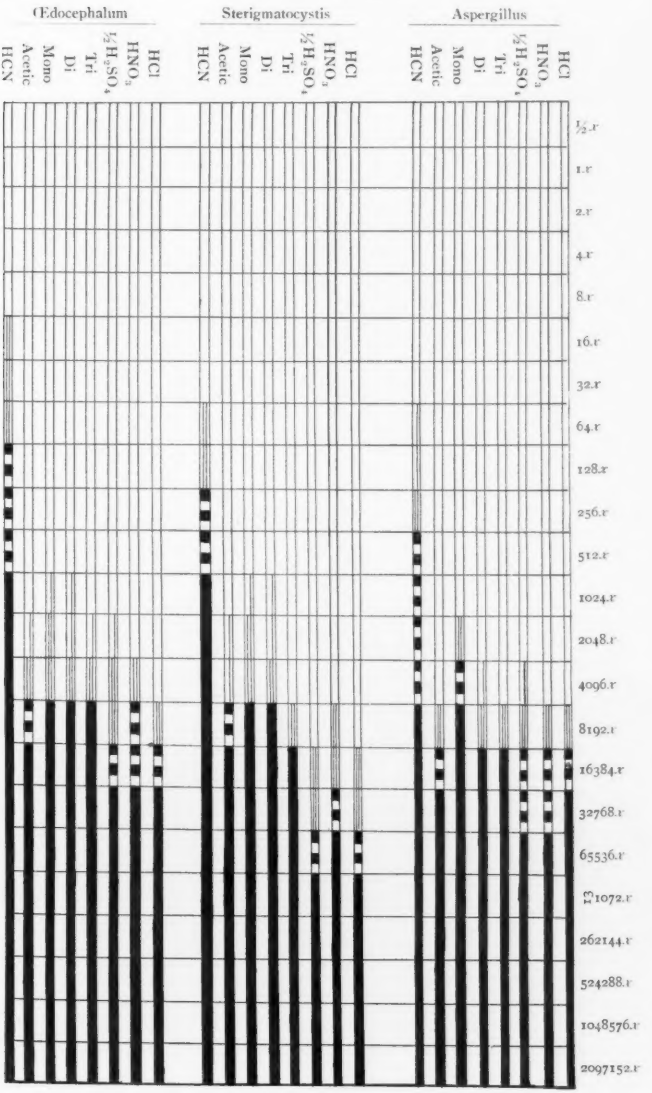
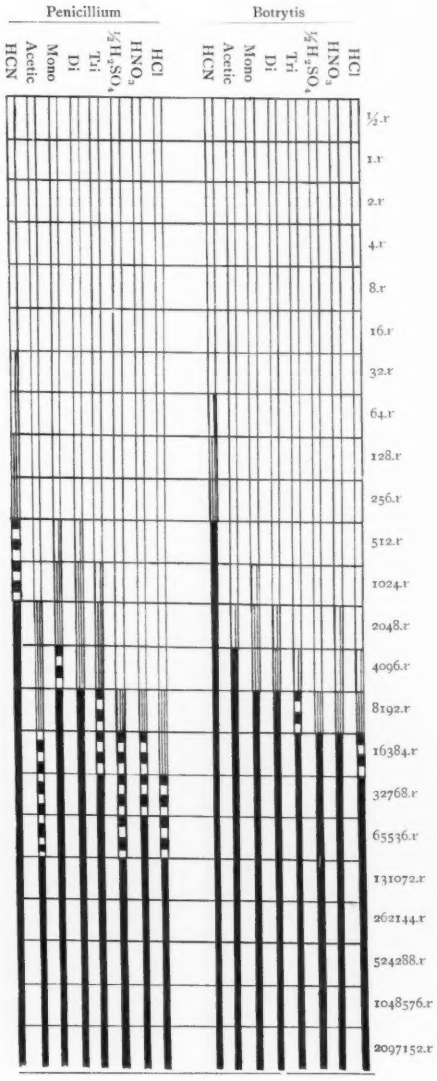

Œdocephalum
Sterigmatocystis
Aspergillus
HCN
Acetic
Mono
Di
Tri
½H2SO4
HNO3
HCl
½ r
1 r
2 r
4 r
8 r
16 r
32 r
64 r
128 r
256 r
512 r
1024 r
2048 r
4096 r
8192 r
16384 r
32768 r
65536 r
131072 r
262144 r
524288 r
1048576 r
2097152 r

DIAGRAM I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Botrytis
HCN
Acetic
Mono
Di
Tri
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HNO3
HCl
½.r
1.r
2.r
4.r
8.r
16.r
32.r
64.r
128.r
256.r
512.r
1024.r
2048.r
4096.r
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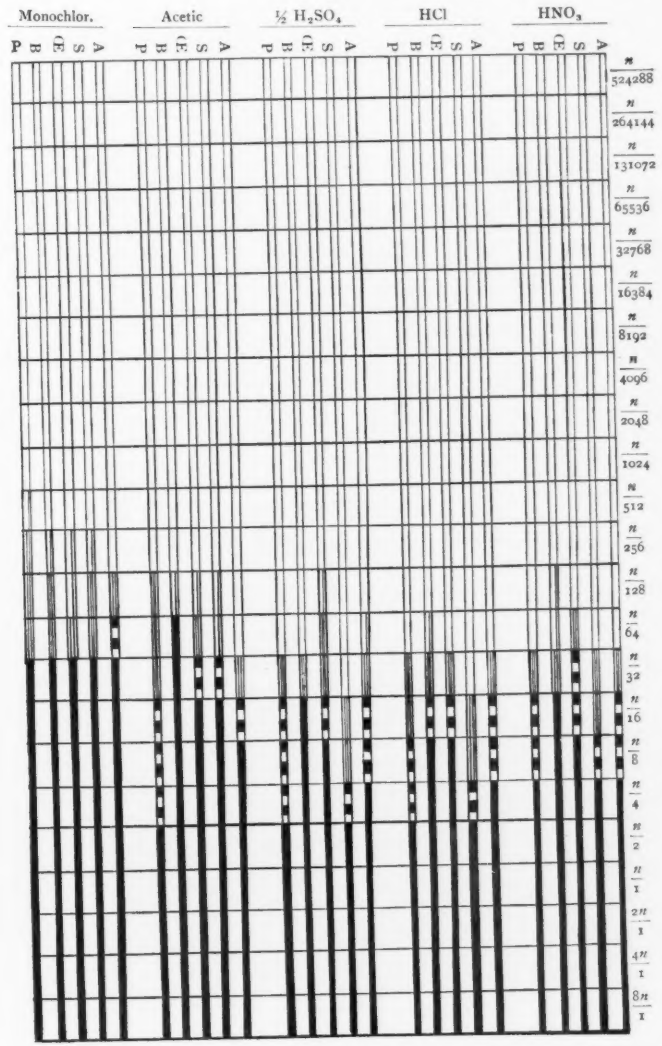
1048576.r
2097152.r

DIAGRAM II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Acetic
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HCl
HNO_3
P B GE S A
n/524288
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n/16384
n/8192
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n/256
n/128
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n/8
n/4
n/2
n/1
2n/1
4n/1
8n/1

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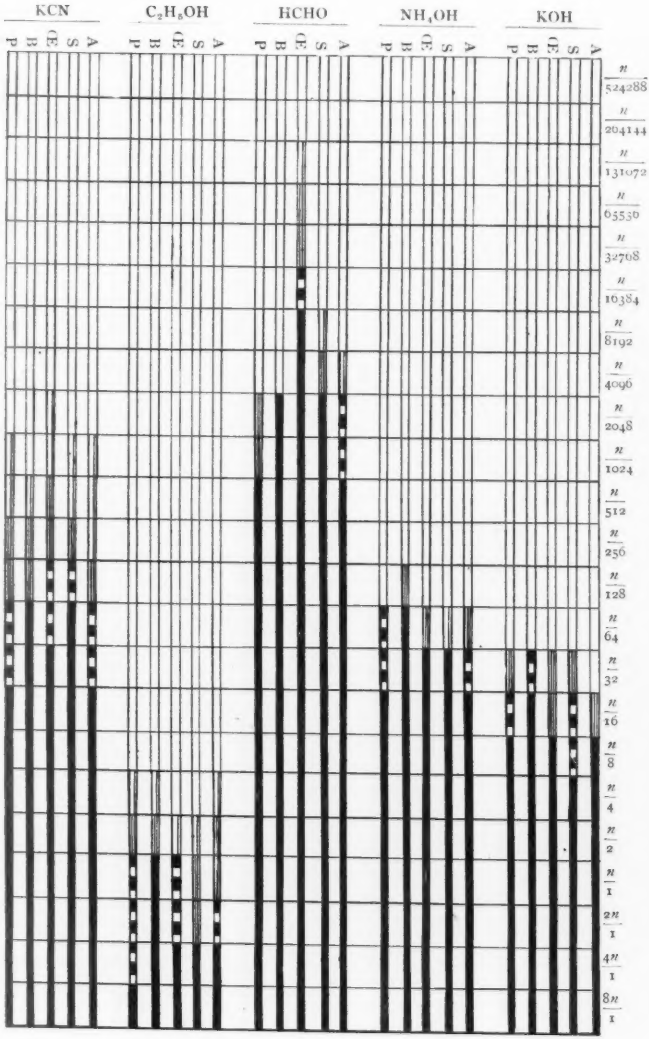
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HCHO
NH_4OH
KOH
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n/8192
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n/256
n/128
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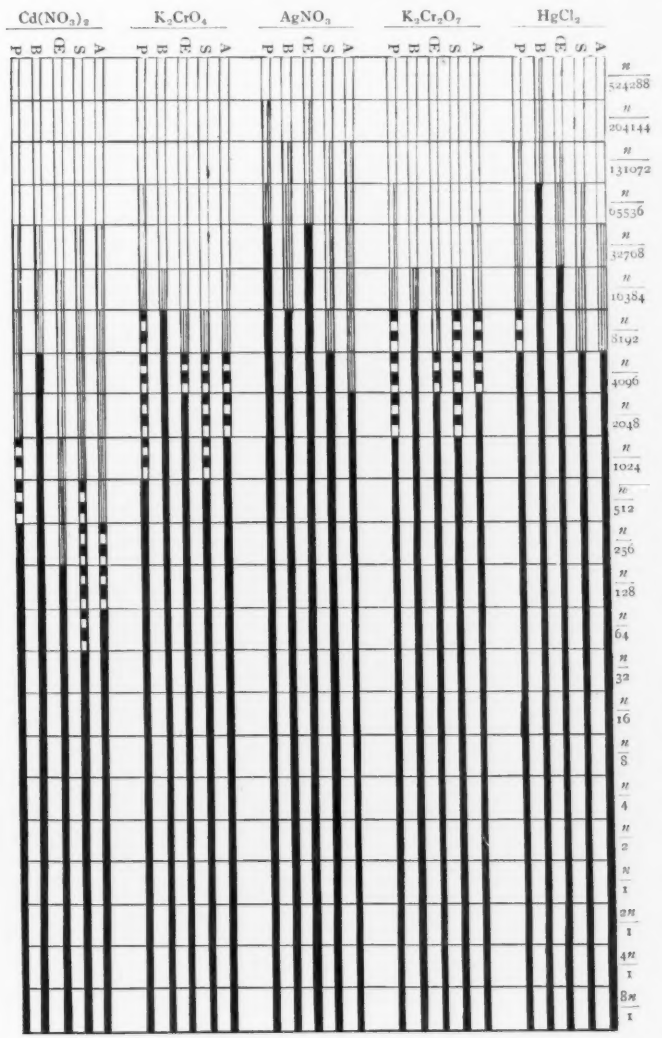
n/8
n/4
n/2
n/1
2n/1
4n/1
8n/1

DIAGRAM IV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K_2CrO_4
$AgNO_3$
$K_2Cr_2O_7$
$HgCl_2$
P B E S A
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8n/1

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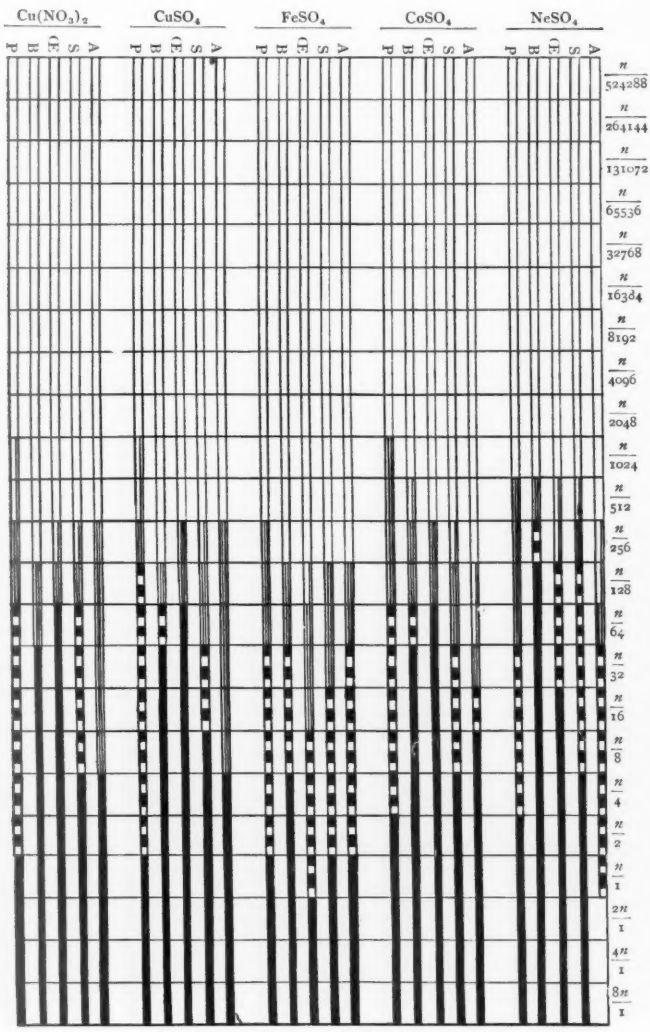

Cu(NO3)2
CuSO4
FeSO4
CoSO4
NeSO4
P
B
(E)
S
A
n/524288
n/264144
n/131072
n/65536
n/32768
n/16384
n/8192
n/4096
n/2048
n/1024
n/512
n/256
n/128
n/64
n/32
n/16
n/8
n/4
n/2
n/1
2n/1
4n/1
8n/1

DIAGRAM VI

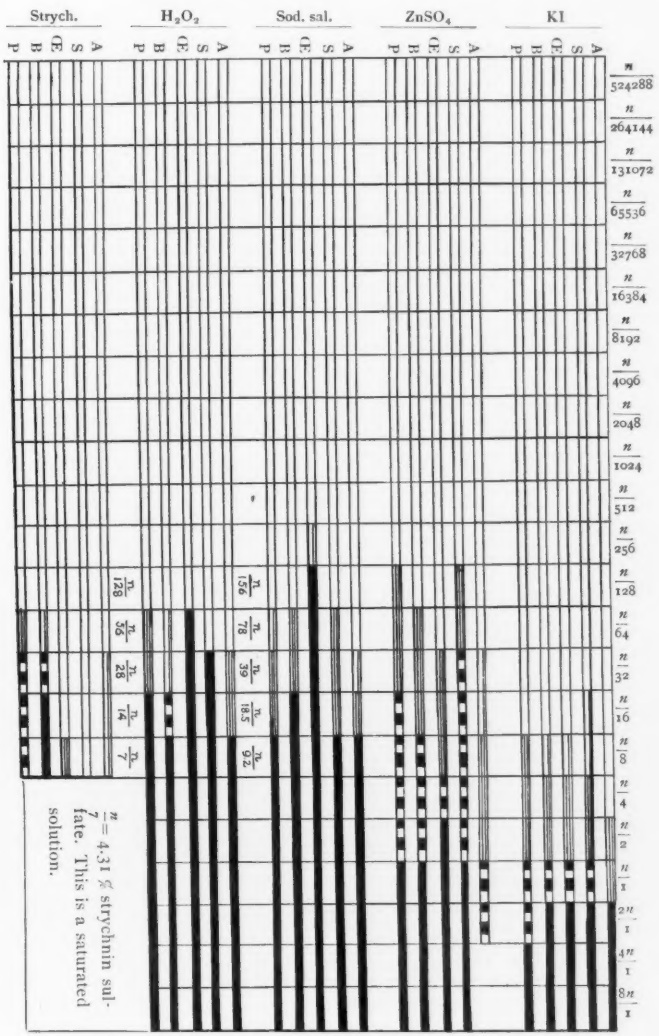

Strych.
H_2O_2
Sod. sal.
$ZnSO_4$
KI
P B GE S A
$\frac{n}{524288}$
$\frac{n}{262144}$
$\frac{n}{131072}$
$\frac{n}{65536}$
$\frac{n}{32768}$
$\frac{n}{16384}$
$\frac{n}{8192}$
$\frac{n}{4096}$
$\frac{n}{2048}$
$\frac{n}{1024}$
$\frac{n}{512}$
$\frac{n}{256}$
$\frac{n}{128}$
$\frac{n}{64}$
$\frac{n}{32}$
$\frac{n}{16}$
$\frac{n}{8}$
$\frac{n}{4}$
$\frac{n}{2}$
$\frac{n}{1}$
$\frac{2n}{1}$
$\frac{4n}{1}$
$\frac{8n}{1}$
$\frac{n}{128}$ $\frac{n}{35}$ $\frac{n}{28}$ $\frac{n}{14}$ $\frac{n}{7}$
$\frac{n}{156}$ $\frac{n}{78}$ $\frac{n}{39}$ $\frac{n}{18.5}$ $\frac{n}{9.2}$
$\frac{n}{7}$ = 4.31 % strychnin sulfate. This is a saturated solution.

DIAGRAM VII

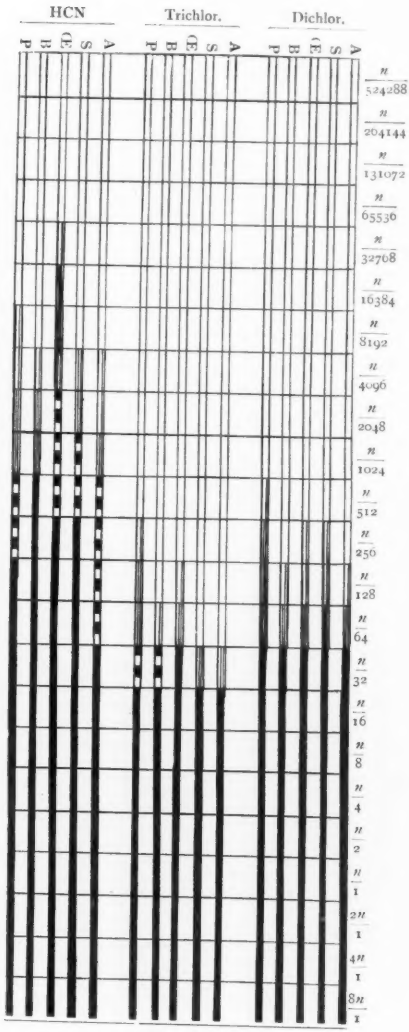


DIAGRAM VIII

compared with the checks; while $\frac{n}{8}$, the limiting culture, failed to fruit in six days.

With HCl a sixth form, *Rhizopus nigricans*, was tested. Like its near relatives the mucors, it makes very unsatisfactory growth in a liquid medium, and was therefore discarded. Its resistance to HCl proved to be practically the same as that of Œdocephalum. The limiting culture of Aspergillus, $\frac{n}{32}$, developed a number of abnormal conidiophores, each bearing 1–4 sterigmata, on the sixth day.

In the case of HCl, as in the case of all other acids tested, an attempt was made to determine the relative toxic value of the anion. The potassium salt was used. This salt becomes highly ionized into $\overset{+}{K}$ and $\overline{Cl}$ ions in moderately concentrated solutions. In a $\frac{n}{4}$ KCl solution 84 per cent. of the salt is thus ionized. In a similar concentration of HCl 89 per cent. is ionized into $\overset{+}{H}$ and $\overline{Cl}$ ions. (Kohlrausch, '85.) In the former we have $\overset{+}{K}$ and $\overline{Cl}$ ions and some KCl; in the latter, $\overset{+}{H}$ and $\overline{Cl}$ ions and some HCl. It is evident therefore that the essential difference between these solutions is the replacing of $\overset{+}{H}$ in the latter by $\overset{+}{K}$, and the HCl by KCl in the un-ionized portion. The significant point is that the *concentration of $\overline{Cl}$ ions is practically the same in both.* The $\frac{n}{4}$ HCl solution is fatal to the spores of Aspergillus, while $\frac{n}{4}$ KCl solution has practically *no injurious influence.* Indeed, this fungus germinates, grows, and fruits normally in a solution of double this concentration of KCl. Hence we know that the $\overline{Cl}$ ion is *relatively harmless* to this mold. Similar tests proved its low toxic value to all the molds used.

The absolute toxic value of ionic $\overline{Cl}$, and other weakly toxic anions, is, however, very difficult to determine. In this connection it should be remembered that the potassium salts of the various acids are, although highly, far from being completely ionized at the concentrations permitting germination of mold

spores. Then, too, we are, perhaps, not justified in assuming that ionic $\overset{+}{K}$ is entirely non-toxic; for although potassium is a necessary food element for all plants, may it not be that great concentrations of even so good a thing as ionic $\overset{+}{K}$ may be bad for fungi? Later, this will be shown to be the case with iron, an element which, while absolutely necessary in small quantities for all plants (Molisch, '94), is quite toxic in excess for both fungi and higher plants.

Experiments with Aspergillus and Œdocephalum in KCl solutions, however, enable us to say quite positively that for these forms the anion $\overset{-}{Cl}$ has at most *less* than one thirty-second the toxic value of ionic $\overset{+}{H}$, and may therefore be disregarded in a discussion of the toxic properties of HCl.

Nitric acid, HNO_3; 48, *141*, 384. Ionized in almost the same proportion as HCl (Kohlrausch, '85), HNO_3 proved much more toxic. Inasmuch as the concentration of $\overset{+}{H}$ ions is practically the same as in HCl, and the $\overset{-}{NO}_3$ ion is practically non-toxic, having a toxic value of less than one thirty-second that of ionic $\overset{+}{H}$, we must look for an explanation in the toxic value of the un-ionized molecule, HNO_3. This was found to be approximately 7.7 times that of ionic $\overset{+}{H}$[2]. In other words a molecule of HNO_3 loses nearly seven eighths of its toxic properties for molds on becoming ionized. Krönig and Paul ('97) found that anthrax spores immersed in a $\frac{n}{1}$ solution of HNO_3 for two hours were entirely destroyed. A similar immersion of a similar preparation of spores in the same concentration of HCl permitted the survival of 385 colonies. Preparations of spores immersed in $\frac{n}{16}$ solutions of these acids, however, showed far *less* variation in toxic properties, although the HNO_3 was still distinctly more toxic. This was evidently due to the fact that at the latter concentration the acids were both much more highly ionized,

[2] See table I, p. 325.

hence both more nearly approximated the toxic value of ionic $\overset{+}{H}$. This toxic power of the un-ionized molecule these workers termed its "specific poisonous effect."

According to this reasoning, then, were HCl and HNO_3 both completely ionized we should expect to find them equally toxic. This matter has been fully tested by Kahlenberg and True ('96). They found that toward Lupinus which is killed by a $\frac{n}{3200}$ concentration of ionic $\overset{+}{H}$, they and all other completely ionized acids with non-toxic anions had *exactly the same toxic value*.

The influence of HNO_3 on germination was much the same as that of HCl, $\frac{n}{1024}$ causing distinct retardation of both germination and early growth. Later, however, a marked difference in the appearance of the cultures manifested itself. Cultures containing the HNO_3 in $\frac{n}{32}$ to $\frac{n}{256}$ concentration produced in nearly every case a much heavier mycelium than in the corresponding cultures in HCl. Fruiting was retarded, but not so greatly retarded as was usually the case where mycelial development was so strongly stimulated.

The stimulation of mycelial development was possibly due to the non-toxic nitrogenous $\bar{N}O_3$ ion. Cultures of Aspergillus and Œdocephalum in $\frac{n}{1}$ solutions of KNO_3, however, did not establish this view, as they did not greatly differ from those in similar concentrations of KCl and K_2SO_4, although the concentration of $\bar{N}O_3$ ions would in this case be some forty times as great as in the cultures of HNO_3 showing the most stimulation. A more likely proposition is that it was due to the same factor as the increased toxicity, viz., the un-ionized molecules. The fact that the general appearance of the cultures resembled that of cultures injured by the oxidizing poisons considered later would suggest that power of the nitric acid molecule as being the active influence. The fact that fruiting was not greatly retarded, considering the abnormal mycelial development, is in harmony with this suggestion.

Sulfuric acid, ½ H_2SO_4; 61, *205*, 589. As will be observed, $\frac{n}{1}$ of this agent is based on the half molecule in order to make it strictly comparable with the other acids, the others being all monobasic.

H_2SO_4 becomes ionized first into $\overset{+}{H}$ and $\bar{H}SO_4$ ions, but as dilution increases the $\bar{H}SO_4$ ion further breaks up into $\overset{+}{H}$ and $\bar{\bar{S}O_4}$. The ionization of H_2SO_4 at the average inhibiting point is about 62 per cent. only (Kohlrausch '85). Each 100 molecules, then at this concentration breaks up into approximately 124 $\overset{+}{H}$, 76 $\bar{H}SO_4$ and 24 $\bar{\bar{S}O_4}$ ions. This solution having a greater toxic value than a similar concentration of ionic $\overset{+}{H}$ and the anion being practically non-toxic, the excess of toxic properties must be due to the partially ionized group $\bar{H}SO_4$. By referring to table I it will be seen that the toxic value of this anion is approximately 1.3 in terms of ionic $\overset{+}{H}$.

In this as in the other mineral acids, Sterigmatocystis proved the most resistant, $\frac{n}{2}$ being necessary to kill. Botrytis was the most easily killed, $\frac{n}{16}$ being fatal. Œdocephalum, although requiring $\frac{n}{8}$ concentration to kill the spores, was considerably injured by $\frac{n}{128}$, greatly injured by $\frac{n}{64}$, and produced a very light yeast-like mycelium in $\frac{n}{32}$, which on the third day practically ceased growing. On the whole, H_2SO_4 retarded germination less than HCl and HNO_3.

Acetic acid, CH_3COOH; 25.6, *83*, 314. This acid at the inhibiting point, $\frac{n}{24}$ is but 2 per cent. ionized (Kohlrausch '85). The toxic value of the anion was found to be about $\frac{1}{13}$ $\overset{+}{H}$, but as so small a proportion of the acid is ionized the influence of the anion may be disregarded. The toxic properties of this acid are

therefore to be attributed almost wholly to the un-ionized molecule, CH_3COOH, which proves to have a toxic value of 2.8.$\overset{+}{H}$.

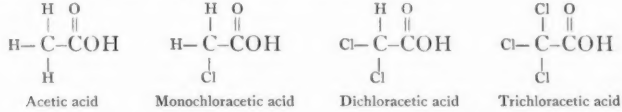
Penicillium showed a marked specific resistance to this acid, requiring $\frac{n}{2}$ for 48 hours to kill. This observation was accepted only after repeated trials. Sterigmatocystis, so resistant to the mineral acids, succumbed to $\frac{n}{16}$, and $\frac{n}{32}$ inhibited germination. Botrytis was particularly susceptible, being killed by $\frac{n}{64}$, which is but one eighth the strength of HCl required for the same result. Although so much more fatal to Sterigmatocystis and Botrytis, germination and early mycelial development was much less retarded than with the mineral acids; $\frac{n}{256}$ retarded the germination of Œdocephalum, but in the other forms this concentration had little effect.

That acetic acid should prove so much more toxic to fungi than the mineral acids was not anticipated (Migula '90). Heald ('96) found that it had but one eighth the toxic value of $\overset{+}{H}$ on Zea, and one fourth on Pisum. Kahlenberg and True ('96) found about the same relation with Lupinus. The great variation in protoplasmic resistance to this acid is well shown by the following data: the vinegar eel, *Rhabditis aceti*, thrives in a $\frac{n}{2}$ solution, which is the fatal concentration for Penicillium. Aspergillus spores are killed by $\frac{n}{8}$, those of Sterigmatocystis and Œdocephalum by $\frac{n}{16}$, and those of Botrytis by $\frac{n}{64}$. $\frac{n}{200}$ is fatal to Zea, and $\frac{n}{1600}$ to Pisum.

It is of interest to note in this connection that not only are great differences to be expected between different organisms in their resistance to deleterious agents, but different individuals of the same genus and even of the same species may have very different powers of resistance, depending largely no doubt on previous environment. Pfeffer ('95) grew Aspergillus on a nutritive medium containing 8 per cent. dextrose and 1 per cent. acetic acid, and found that the fungus assimilated a far larger amount

of acid than the dextrose. It will be noted that $\frac{n}{8}$ (0.7 per cent.) acetic acid proved fatal to the spores of Aspergillus used in this study, and in a 0.17 per cent. solution less than 1 per cent. germinated.

The chloracetic acids.—These acids are formed by the replacement of one, two, and three atoms of H, respectively, in the acetic acid radical by the element Cl; thus —



It is a rule that the halogen substitution-products of carbon compounds have a toxic value which bears a close relation to the number of H atoms in the organic radical or hydrocarbon which have been replaced by the halogen. To quote from Davenport ('97): "Beginning with methane, CH_4, we find this substance — marsh gas — innocuous when mingled with air. As the H atoms become replaced by one or more Cl atoms, the poisonous properties increase,—

CH_3Cl is slightly anesthetic,

$CHCl_3$ = chloroform,

CCl_4 is very dangerous, stupefying involuntary muscles." Many such examples might be quoted, establishing this rule. It was a matter, then, of great surprise to find what, at first sight, seemed to be a direct exception to this rule in the action of the chloracetic acids on the mold fungi. Their critical points were determined as follows:

(Acetic acid - - - -	25.6	*83*	314)
Monochloracetic acid - -	8.8	*58*	64
Dichloracetic acid - - -	10.4	*64*	64
Trichloracetic acid - -	37	*90*	115

As soon as the experiment was complete the stock solutions used were placed in the hands of a chemist who formed the potassium salt of each by just neutralizing with KOH. These salts are all quite highly ionized, the cathion being ionic $\overset{+}{K}$ and the anion the acid radicals of the respective acids. The toxic

values of these anions, as nearly as could be determined with Aspergillus and Œdocephalum, were as follows:

Monochloracetic - - - -	$-CH_2ClCOO$,	$\frac{1}{32}$ $\overset{+}{H}$
Dichloracetic - - - -	$-CHCl_2COO$,	$\frac{1}{21}$ $\overset{+}{H}$
Trichloracetic - - - - -	$-CCl_3COO$,	$\frac{1}{11}$ $\overset{+}{H}$

Here the toxic values, although not high, are in the expected order.

Let us next glance at the toxic properties of these acids in a practically completely ionized condition. Kahlenberg and True ('96) found that with Lupinus $\frac{n}{6400}$ monochloracetic permitted growth. The same concentration of dichloracetic was evidently on the line, one plant being killed by it while the check survived. $\frac{n}{6400}$ trichloracetic proved fatal. Ionization being practically complete at this great dilution, we have to deal with ionic $\overset{+}{H}$ and the anions only. The toxic values of such solutions should be equal to that of HCl + the value of the anion. This is exactly what we find. Monochloracetic with its non-toxic anion has the same toxic value as HCl for Lupinus. The others are somewhat more toxic; due doubtless to the influence of the anions, which are apparently relatively more toxic towards the higher plants than towards the molds.

At the concentrations at which they are effective towards the fungi, however, we have an entirely different condition as regards ionization, as may be seen from the following:

Monochloracetic inhibits at	$\frac{n}{35}$	and is	20	per cent.	ionized.
Dichloracetic "	$\frac{n}{32}$	"	70	"	"
Trichloracetic "	$\frac{n}{22.8}$	"	88.8	"	"

This variation in the ionization (Ostwald '89) is the key to the explanation of this apparent deviation from the rule referred to above.

The introduction of Cl into the acetic radical results in two distinct changes in the chemical and physical properties of the acid. (1) A great increase in the toxic properties of the un-ionized molecules. (2) A great increase in the ionization of

the acid in aqueous solution of given concentration. That the great ionization often masks the effect of the increase in the toxic properties of the un-ionized part is easily understood when we recall that the toxic value of the ionized portion of an acid is not greater than the toxic value of the ionic $\overset{+}{H}$ + the toxic value of the anion.

Putting all together, we get in brief the following: (1) The replacement of one H in the acetic radical by Cl doubles the toxic properties of the un-ionized portion, and increases the ionization in a $\frac{n}{35}$ solution from 2 per cent. to 20 per cent.; the resultant of these two factors is an increase in the toxic value of about 40 per cent. in a $\frac{n}{35}$ concentration. (2) The replacement of two atoms of H by Cl more than trebles the toxicity of the un-ionized molecule and causes ionization to advance from 2 per cent. to 70 per cent. in a $\frac{n}{32}$ solution. This gives a net increase of about 30 per cent. in toxic properties at this concentration. (3) When all three H atoms are thus replaced by Cl the un-ionized molecule has a toxic value of over five times its original value and ionization advances from 2 per cent. to 88.8 per cent. in a $\frac{n}{22.8}$ solution. At this concentration the effect of the greater ionization is *more* than can be made up by the increased toxic properties of the 11.2 per cent. remaining in molecular form, hence a fall in the toxic properties of this acid at $\frac{n}{22.8}$ concentration as compared with the original acetic.

The values of the un-ionized molecules as worked out in table I, p. 325, show that these acids are very far from being an exception to the rule that the toxic properties of compounds increase with the introduction of Cl into the organic radical. These values are as follows:

Acetic acid,	2.8	times	the value of ionic	$\overset{+}{H}$.
Monochloracetic acid,	4.7	"	"	"
Dichloracetic acid,	9.5	"	"	"
Trichloracetic acid,	14.1	"	"	"

The addition of any highly ionized acid to a solution of a weaker acid throws back the ionization of both, but more particularly that of the one having the low ionization. This method of decreasing the ionization, and thereby increasing the toxic properties, was used with di- and trichloracetic acids. To solutions of these acids HCl was added — molecule for molecule — and the resulting mixture was tested with the five molds. The *calculated* increase of toxic properties due to the *forcing back of the ionization* of dichloracetic acid was found to be 168 units. The experimental test gave an increase of toxicity for the mixture of 147 units more than the *additive* toxic properties of the two acids mixed. In the case of trichloric acid the experimental test gave a slight excess (13 units) over the calculated increase.[3]

Krönig and Paul's ('97) work on anthrax spores is of especial interest here. A preparation of anthrax spores immersed in a $\frac{n}{1}$ solution of trichloracetic acid for 2 hours and afterwards cultured in a favorable medium proved to be entirely sterilized, not a spore surviving. In another test when a similar preparation of spores was immersed in the same concentration for 20 minutes, comparatively few survived. Tests were also made with the same acid at $\frac{n}{16}$ concentration. Spores immersed in this concentration for 56 hours showed much less injury than those immersed for 20 minutes in $\frac{n}{1}$ solution, thus showing clearly that the *efficacy of the acid as a disinfectant was more greatly reduced than could be accounted for by dilution only*. Their work on the other acetic acids, although not extensive, is quite in harmony with the results here recorded for the molds.

Hydrocyanic acid, HCN; 0.36, *3*, 20. This poison, so deadly to the higher vertebrates, has long been known to be much less toxic to less highly organized structures. Extremely fatal to man even in minute quantities, the more lowly organized

[3] For details of these tests and other data regarding the relation of electrolytic dissociation to the physiological action of acids, see article by the writer in Journal of Physical Chemistry **3**:263. May 1899.

Ascaris resists a 3 per cent. solution for 75 minutes. A myriapod (Fontaria) excretes HCN when irritated! The accumulation of many such data has led to the general acceptance of the theory that HCN acts chiefly or wholly upon the aldehydes of the nerve centers (Loew '93). This is no doubt quite satisfactory from the point of view of the animal physiologist, but it leaves us without any explanation for its violent toxic properties toward plants, which have no nerve centers.

Little work seems to have been done with it on plants. Kahlenberg and True ('96) found that toward Lupinus it had double the toxic value of ionic $\overset{+}{H}$, $\frac{n}{6400}$ proving fatal. To the molds, however, it is relatively a much more powerful agent, having 76.6 times the value of $\overset{+}{H}$, thus ranking as one of their most fatal poisons. The data on the ionization of this acid are meager. At $\frac{n}{32}$ it gives about one sixteenth the electrical conductivity of acetic acid at the same concentration (Ostwald '85). From this we would judge that the ionization is practically zero at the concentrations with which we have to deal.

The value of the $\overline{C}N$ ion was determined by means of the potassium salt, which is quite highly ionized (Kohlrausch '79). It was found to be approximately $8\overset{+}{H}$ for the molds. Were HCN fully ionized we would expect its solutions to have a toxic value of about $9\overset{+}{H}$. The fact that the practically un-ionized solutions with which we deal have a value of over *eight times* that calculated for the entirely ionized acid tells for the extremely toxic influence of the un-ionized molecule, HCN.

Aspergillus showed a high specific resistance to this agent, $\frac{n}{32}$ being necessary to kill all the spores. Œdocephalum was particularly sensitive, being injured by $\frac{n}{32768}$, inhibited by $\frac{n}{2048}$, and killed by $\frac{n}{256}$.

In table I the toxic values of the un-ionized molecules of the

TABLE I.

RELATIVE TOXIC PROPERTIES OF UN-IONIZED MOLECULES.

I. As measured by their inhibiting powers.

HCN	$\frac{1}{2}H_2SO_4$	HNO_3	Trich.	Dich.	Monoch.	Acetic	HCl.	Agent	
$\frac{n}{683}$	$\frac{n}{10}$	$\frac{n}{14.5}$	$\frac{n}{22.8}$	$\frac{n}{32}$	$\frac{n}{35}$	$\frac{n}{24}$	$\frac{n}{8.8}$	Concentration inhibiting germination	
7666.	112	163	255	359	396	277	100	Relative toxic value. HCl = 100	
—	62	90.6	88.8	70	20	2		Per cent. ionized at inhibiting point	
—	62	90.6	88.8	70	20	2		Toxic value of cathion in terms of ionic $\dot{H}$	Ionized
—	—	—	8	3.3	.6	—		Toxic value of anion, ditto	
—	62	90.6	96.8	73.3	20 6	2		Total, cathion + anion	
7666	50	72.4	158.2	285.7	375.4	275		Residual units	Un-ionized
100	38	9.4	11.2	30	80	98		Per cent. un-ionized	
76.6	*1.3*	*7.7*	*14.1*	*9.5*	*4.7*	*2.8*		*Value of ionized molecules in terms of ionic $\dot{H}$*	

TABLE I.—(*Continued.*)

RELATIVE TOXIC PROPERTIES OF UN-IONIZED MOLECULES.

II. As measured by their killing powers.

HCN	$\frac{1}{2}H_2SO_4$	HNO_3	Trich.	Dich.	Monoch.	Acetic	HCl.	Agent	
$\frac{n}{102}$	$\frac{n}{3.5}$	$\frac{n}{5.3}$	$\frac{n}{18}$	$\frac{n}{32}$	$\frac{n}{32}$	$\frac{n}{6.5}$	$\frac{n}{3.3}$	Concentration killing spores	
3070	104	160	534	960	960	196	100	Relative toxic value. HCl = 100	
—	54.	90	88	70	19.9	1		Per cent. ionized at death-point	
—	54	90	88.	70	19.9	1		Toxic value of cathion in H	Ionized
—	—	—	8	3.3	.5	—		Toxic value of anion in H	Ionized
—	54	90	96.	73.3	20.4	1		Total cathion + anion	Ionized
3070	50	70	438	887	940	195		Residual units	Un-ionized
100	46	10	12	30	80.1	99		Per cent. un-ionized	Un-ionized
30.7	*1.1*	7	*36.5*	*29.6*	*11.7*	*1.97*		*Value of un-ionized molecules in terms of ionic* $\overset{+}{H}$	Un-ionized

different acids, as shown by their inhibiting and killing powers toward the spores of the five molds, are approximated. Too much importance must not be attached to the *exact numerical* value here expressed for the different molecules, for several reasons. We do not know the reaction of the acids toward the nutrient medium in which we grow our plants; we do not know the exact effect of the salts and sugars present on the ionization of the acids; and were it possible to eliminate every factor causing doubt or error, we should undoubtedly find the relative toxic properties of the molecules varying with almost every plant tested. Let me repeat: the exact numerical values here given are *not* significant. The order and general proportions of these values *are* significant. The emphasis is laid on part I of this table for reasons already given.

Line 1 gives the strength of the various acids required on an average to inhibit germination. Line 2 is developed from line 1. It gives the relative toxic properties of the acids expressed numerically, HCl being taken as 100 for a basis of comparison. The ionized and un-ionized portions are considered separately, lines 4, 5, and 6 being devoted to the former, and 7, 8, and 9 to the latter. Line 4 gives the toxic value of the cathion in units of ionic $\overset{+}{H}$; line 5 that of the anion. Line 6, being the total of 4 and 5, gives that portion of the total toxic value of the acid which is to be attributed to ionized portion. Line 7 gives the "residual units." In other words, that part of the total toxic value to be accounted for by the un-ionized portion. Line 8 gives the percentage of such un-ionized molecules present at the inhibiting point. Line 9, the quotient of the residual units divided by the percentage of un-ionized molecules, gives in terms of ionic $\overset{+}{H}$ the toxic value of the different acid molecules.

Part II is worked out similarly, and has reference to the toxicity of the acids towards the molds, as shown by their power to *kill* the spores.

[*To be concluded.*]

THE DEVELOPMENT OF THE MICROSPORANGIUM AND MICROSPORES IN CONVALLARIA AND POTAMOGETON.

KARL M. WIEGAND.

(WITH PLATES XXIV–XXV)

DURING the past decade perhaps no one portion of the field of botany has been worked upon so much as that which deals directly with the organs concerned in the sexual process. Especially is this true of the higher plants, but a careful survey of the present condition of our knowledge on this very point shows that some of the most vital questions have as yet received no solution. The researches of such men as Hofmeister, Strasburger, Guignard, and Warming have discovered facts in regard to the ovule, the embryo-sac, and the cell which have already become so universally known as to need no further mention here. But we still know almost nothing about the essential significance of some phenomena of most common occurrence, and many questions have as yet been investigated only in connection with so few plants that generalizations are extremely unsafe. It was principally with the hope of increasing, if only by a few species, the range of observations that the present studies were undertaken.

The choice of subjects signifies very little. It was influenced much more by the necessity of using plants obtainable at certain times rather than by an idea that they all represented different types of structure.

METHODS.

The methods employed in this work differ in no essential way from those so often described in recent cytological works, consequently it is scarcely necessary to repeat them again in detail. The following general statements are intended rather for those who wish simply to know the stains, fixing agents, etc., chosen for the work.

The Flemming chrom-osmo-acetic acid solution has of late years proved of such value that it must now be regarded as the very best fixing agent for cytological work. For the following studies material from no other fixing agent was used, although some was put up in alcohol, sublimate, and picric acid. None of the latter gave satisfactory results. The secret, if there is any, in the use of the Flemming solution seems to lie in obtaining rapid penetration. To accomplish this the material cannot be subdivided too much, and when possible even the anthers themselves should be cut open. From six to twelve hours is sufficient for complete fixation. The black discoloration caused by the osmic acid was removed by the aid of hydrogen peroxid either upon the sections themselves or preferably upon the material *in toto*.

For clearing, cedar-wood oil gave the best results. It was always added with great care in order to avoid too rapid change of density, otherwise collapse of the cells often occurred. The paraffin used possessed a melting point of 54°. This also was added with the same degree of care. The sections ranged in thickness from four to six and two thirds microns, and were all cut on a Minot-Zimmerman revolving microtome.

Considerable experimentation was necessary before a suitable staining method could be devised. Among others, Rosens' fuchsin-methylene blue method,[1] iron-hæmatoxylin and the Flemming safranin-gentian-violet-orange combination were the most important. The latter was at length almost exclusively employed, and was found very satisfactory. As a general nuclear stain, however, better results were obtained by the omission of the safranin, and at the same time a large amount of time was saved. The orange G was always used in a very dilute solution, and for a very short time, from 15 sec. to 2 min. giving the best results. For a chromosome stain the gentian-violet was allowed to act for a short time only, but for differentiating the spindle fibers and kinoplasmic radiations, much better results were obtained with a very weak solution (2–3 drops of the stock

[1] Beiträge zur Kenntniss der Pflanzenzellen. Cohn's Beiträge 5:443. 1892.

solution made according to Lee in 100^{cc} of water) acting for from 12 to 24 hours. After washing out in absolute alcohol, further differentiation was obtained with clove-oil, or in the case of Potamogeton, preferably with anilin oil. The action was then stopped with bergamot oil before mounting in balsam.

CONVALLARIA MAJALIS L.

The Liliaceæ have so far furnished some of the very best subjects for cytological study. To those already studied must now be added another good type, namely Convallaria. This plant has unfortunately up to the present time received very little attention, although the large nuclei and long rod-like chromosomes almost equal Lilium in the ease with which they may be studied. The published observations are at present limited to those of Strasburger on the pollen of *C. Polygonatum*, and on the endosperm of *C. majalis*.[3] The observations on both of these are very brief.

The material for this study was obtained from plants grown in the University greenhouses. They were here under a constant though moderate condition of forcing, which thus enabled one to obtain an unusually large proportion of dividing nuclei. The progressive development of the flowers in the raceme makes it possible to find many stages on a plant all at one time. To insure rapid penetration of the fixing agent the upper and lower ends of each bud were cut away, thus exposing directly the cells of the anther

In order to determine if there might not be a relation between the nuclear division and the environment of the plant, especially as to the amount of light, humidity, etc., several experiments were made. In the case of Convallaria only the effect of light could be studied, since the other conditions in the greenhouse were practically the same. In order to test the effect of light, material was collected at various times during the day and night. The results, however, were wholly negative. Spindles were found in about the same proportion in every collection.

[2] Befruchtungsvorgänge bei den Phanerogamen 171. 1884.

[3] Theilungsvorgänge der Zellkerne 43. 1882.

THE DEVELOPMENT OF THE MICROSPORANGIUM.

In 1873 Warming's important work on the development of the anther apeared.[4] In this paper we find for the first time a correct description of the succession of cell divisions resulting in the formation of the archesporium and the anther wall. It was not followed up, however, by other investigators, and even in late years nothing of importance has been done along this line. The most important of the subsequent papers is undoubtedly that of Engler in which Orchis is taken up in detail.

Warming established the fact that the archesporium arises from the daughter cells resulting from the division of the hypodermal layer at each corner of the anther. If the hypodermal cells form a true layer, then the archesporium will usually also be in the form of a layer; but in some cases the hypodermal cells may be reduced to one, and the resulting archesporium in that case is simply a vertical row of cells. In any event, there is almost no subsequent division in the archesporium, growth being confined entirely to an increase in size of the existing cells. Of the two original daughter cells of any hypodermal cell, the inner gives rise to the archesporium, the outer to the anther wall. By two or three periclinal divisions progressing in a centrifugal manner a radial row of cells is formed, the inner cell of which becomes the tapetum, the outer the endothecial layer, and the rest finally disintegrate. Although many of the divisions are periclinal, some are radial and others transverse, by which means accommodation is provided for the growth of the archesporium. He finds also that cell division in the epidermis is almost entirely radial and transverse, and this structure remains always one-cell thick. His subjects for investigation, however, included only one monocotyledon; and Engler,[5] who attempted to show more clearly the application of Warming's laws to the monocotyledons,

[4] Untersuchungen über pollenbildende Phyllome und Caulome. Hanstein's Bot. Abh. **2**: 1. 1873.

[5] Beiträge zur Kenntniss der Antherenbildung. Pringsh. Jahrb. f. wiss. Bot. **10**: 275. 1876.

used Orchis as the only type. At present, therefore, our knowledge of the details in this group is very meager.[6]

In Convallaria the early stages in the development of the anther are not so easily understood as are those of the dicotyledons; but after the investigation of a large number of preparations it seems probable that the following is the proper interpretation, not alone for Convallaria but also for many other monocotyledons. The earliest stages obtained show in cross-section a four-angled anther with a radial row of cells at each angle, which apparently result from the division of a single hypodermal cell.

The condition at this stage is represented in *fig. 1*. The innermost cell, the primary archesporial cell, very soon divides in various directions until a considerable mass of tissue is formed. This division takes place very early, so that the final number of archesporial cells is formed even before the anther has become obviously lobed. At this stage it is only with considerable difficulty that the archesporium can be distinguished from the wall (*fig. 2*). The original radial row of cells, descendants of the primary hypodermal cell, may often be recognized for a considerable time after they are first formed, but in some cases one or two cells on either side may also divide several times in a radial direction. The greater portion of the wall, however, is derived from a few irregular divisions of the cells at each side of the archesporium, while the epidermis is at the same time increased by a few anticlinal divisions. In addition to this the cells between the archesporium and the connective may also undergo a few radial divisions. As a result the wall-cells in the older anthers do not stand in distinct radial rows as in the dicotyledons, simply because they were not all derived by radial division; but, notwithstanding this, there are quite

[6] While in press the following papers have appeared:

GUIGNARD, L.— Le développement du pollen et la réduction dans le *Naias major*. Arch. d'anat. Microscop. **2**: 455. 1899.

CALDWELL, O.— Life history of *Lemna minor*. BOT. GAZ. **27**: 37. 1899.

FULLMER, E. L.— The development of the microsporangia and microspores of *Hemerocallis fulva*. BOT. GAZ. **28**: 81. 1899.

regularly three or four layers of cells differentiated entirely around the sporogenous tissue. These stages were found in the autumn previous to the time of flowering, and all subsequent growth, both in the archesporium and in the wall, is due entirely to the increase in diameter of the cells already formed. This was determined by an actual count of the cells in a great many cases.

In most dicotyledonous anthers the archesporium becomes distinct at a very early period. No such sharp demarcation exists, however, in the monocotyledons. In all of the cases studied by the writer, and in those treated by Warming and Engler, the transition from the wall to the archesporium is so gradual, especially in the younger conditions, that only the most careful study enables one to distinguish accurately between them. Although it is difficult to distinguish them solely by their form, it is not so difficult when their cytoplasm is considered. In Convallaria the cytoplasm of the archesporial cells becomes so modified that by its structure alone one can recognize the future mother-cells. The change consists in an increase in the abundance of the cytoplasm, as well as in the formation of a much finer network with scarcely any indication of a granular structure. Taking this into consideration, the wall is now readily seen to consist of four layers.

From the above description it will be seen that there really is here a special case of Warming's law as formulated for the dicotyledons, and not an entirely new process. In other words, the archesporium seems to arise entirely from the division of one or two hypodermal cells rather than from a layer of such cells, and the wall is formed mostly from cells adjacent to the archesporium.

At a slightly later period the fourth or innermost layer of the wall begins to enlarge. The cells grow considerably, and soon might be mistaken for archesporial cells if it was not for the difference in the cytoplasm. This layer is the tapetum (*fig. 3*).

The history of the tapetal nucleus has been studied by

Strasburger in Malva, but not in detail.[7] In this plant two nuclei were found in each cell. Guignard also figured two nuclei in the tapetal cells of Lilium, and it now seems probable that the phenomenon is quite general, as Strasburger in the above cited work states. It was originally believed that these two nuclei were formed by direct division; but Strasburger[8] has shown that this was really a process of fusion, and that the two nuclei were formed at an earlier date by the ordinary indirect method.

In Convallaria, at the period just preceding synapsis, the already enlarged tapetal cells contain only one nucleus; but during synapsis, and even up to the first pollen-mother-cell division, the nuclei one by one divide by the mitotic method. Such spindles are seen in *fig. 4*. It is probable that every tapetal nucleus finally undergoes division, but this could not be accurately determined because of the subsequent fusion. The different stages in the fusion of these nuclei were sufficiently frequent after the first division of the pollen-mother cell (*fig. 5*). Cases could often be found even in anthers where tapetal spindles also occurred. It is doubtful if all the pairs of nuclei fuse — in fact, it is probable that they do not, since many remain distinct even after the tapetum shows signs of disintegration.

The wall of the mature anther in Convallaria presents no new features. It is composed of a conspicuous epidermis, a well-developed fibrous layer (endothecium) with beautiful spiral markings, and the remains of the other wall layer (*fig. 6*). No remnant of the tapetum is now left. The time of disappearance of this structure was interesting because it occurred in some cases as early as the pollen-mother-cell stage, while in others not until after the pollen grains were mature. The time is seldom the same even in anthers from the same bud.

THE NUCLEUS OF THE ARCHESPORIUM.

The very earliest stages of the archesporial nucleus in Convallaria differ only slightly in appearance from true vegetative nuclei (*fig. 7*). The linin thread is exceedingly thin and fine

[7] Ueber den Bau und das Wachsthum der Zellhäute 89. 1882.

[8] Theilungsvorgänge der Zellkerne 99. 1882.

as well as extensive. Careful examination shows that it is not a continuous ribbon at this period, but rather an anastomosing network in which large granules of chromatin of unequal sizes are imbedded, giving it thus a more or less knotty appearance.

At a period quite early in the development of the anther the chromatin network contracts into a dense and ultimately spherical mass, which in most cases is in contact with the nuclear membrane, but may also lie free in the nuclear cavity (*fig. 8*). The ball becomes so dense that it is ordinarily impossible, even with the weakest stains, to distinguish the separate threads of which it is composed, except at the periphery where they project into the nuclear cavity outside. This condition is synapsis in Convallaria. The nucleolus does not lie inside the mass and adjacent to the membrane, as has been claimed for some other plants. On the contrary, it always lies outside so far as could be determined, but usually in contact with the mass and at the side away from the wall more often than toward it.

Judging from the number of preparations obtained, the nucleus must remain in the synapsis condition for a considerable time. The first indication of the return from synapsis is found in the gradual separation of the outermost linin threads, and finally the whole mass becomes more open (*fig. 9*). But a very strange thing now occurs. At the moment when the chromatic thread is spreading out, between its meshes and in the cavity outside are to be seen large granules, or more properly speaking masses of various sizes which are decidedly chromatic, in fact stain exactly like chromatin. The nucleolus meanwhile still remains intact. Where these bodies come from or where they go could not be determined. They were always present, however, at this stage. The masses were often as large as the nucleolus but more often smaller. It might be suggested that in some way the nucleolus becomes fragmented either naturally or by the action of the reagents, but the preparations do not support this view. In very weakly stained nuclei the nearly colorless nucleolus with its central vacuole could be plainly seen, while in the same nucleus the expelled masses were stained

dark like the chromosomes at the time of division. Again it is possible that the chromatin had left the linin thread and collected in the above manner before the material was fixed. Or perhaps the reagents caused such a change in the chromatin. The investigation of fresh material alone can decide whether the process is normal or artificial.

A remarkable change has also taken place in the chromatin thread. Before synapsis it was a network containing very large irregular granules, now it is a spirem with the granules much reduced in size and more uniform. For the most part they are but slightly broader than the scarcely thickened linin. These above mentioned peculiarities were found not in one case alone, but in a great many preparations, in fact in every preparation that contained the right stage.

Synapsis has now been found in many plants, in all of which it always seems to be a natural condition, and it is quite probable that it will prove to be a universal phenomenon, occurring at a certain period previous to each heterotypic division. Besides this, it has now been shown to occur in at least a few animals. Among plants it has been found in the Liliaceæ by Strasburger, Sargant, and others; in the Hepaticae by Farmer; and in Potamogeton and Acorus by the writer, in addition to several other plants, the studies on which have not yet been published. There now seems to be little doubt that the condition is a natural one for the following reasons. It may be found in the fresh material, at least in Convallaria and Lilium. Moreover, it always occurs at the same stage in the development of the anther. Various structural changes in the chromatin thread always accompany it. In addition to this, many preparations show that the mass may be deposited at any side of the nuclear cavity without reference to its position in the anther.

Considerable uncertainty has always existed as to just what synapsis really is. The term was introduced by Moore[9] in the year 1895, but the description was so brief that it is somewhat

[9] Essential similarity of the chromosome reduction in animals and plants. Annals of Botany 9:435. 1895.

difficult to understand just what the author had in mind. It seems probable, however, that the condition found by him was the same as that described above for Convallaria. He was able to demonstrate the appearance of the same phenomenon also in animals, especially in Triton, thus emphasizing the fact that the steps in the maturation of the sexual gametes is to a certain extent similar in both plants and animals.

During the past few years synapsis has been described by several authors, most of whom now consider it to be a natural condition of the cell, although it seems probable that the phenomenon described is not the same in all cases.

Farmer[10] found a contracted condition of the chromatin thread in the spore-mother-cells of the Hepaticae, but the figures do not show as great a contraction as is found in Convallaria. He states that the nuclei at this stage are difficult to fix, often showing signs of fragmentation, and that there is usually a chromatic change in the cell.

Convallaria has so far shown no case similar to that described by Miss Sargant[11] for Lilium, in which two rows of dots were found on the thread before synapsis. A double row in the former plant is found only very late in the history of the spirem; neither does the nuclear membrane disappear or even become indistinct during synapsis. Otherwise the description and figures are quite similar to Convallaria. In some preparations an extrusion of granules from the contracted mass was found, but was interpreted as a fragmentation of the nucleolus rather than as a separation of portions of the chromatin.

The conflicting results obtained are probably due, in part at least, to the fact lately emphasized by Strasburger,[12] that several distinct phenomena have been referred to synapsis. Only one of these is a normal condition, and to this the term synapsis

[10] On spore formation and nuclear division in the Hepaticae. Annals of Botany **9**: 482. 1895.

[11] The formation of the sexual nuclei in *Lilium Martagon*. Annals of Botany **10**: 457. 1896.

[12] Karyokinetische Probleme. Pringsh. Jahrb. f. wiss. bot **28**: 158. 1895.

properly belongs. The others are probably caused by the action of the reagents.

Mottier's[13] figures representing synapsis in Lilium do not show nearly as much contraction as in Convallaria. From the description it would seem also that they represent a much later stage in the development of the spirem than the one in which synapsis occurs, either in Convallaria or in Potamageton.

FIRST NUCLEAR DIVISION OF THE MOTHER-CELL.

The spirem stage preceding division is very well marked in Convallaria (*fig. 10*). The much coiled wire-like thread found immediately after synapsis gradually increases in thickness, and the chromatin granules become less prominent. One large nucleolus and usually two or three smaller ones are present at this period. The longitudinal splitting of the chromatin thread is accomplished so quickly that it was found impossible to observe the successive steps in the process. At the same time the thread becomes considerably thicker than immediately before the division (*fig. 11*). It is difficult to understand just how this doubling takes place, because at the very first indication of such a condition the threads are already separated. They lie parallel with each other and slightly coiled. The further development indicated a more or less complete subsequent fusion of the parts, so that before passing into the nuclear-plate stage the dual nature is entirely lost except for an occasional lobing at either end (*fig. 12*). Not even the granules are longer visible, and the chromosomes are at this stage apparently homogeneous. This is probably only apparent, however, and due really to the density of the stain and the close proximity of the parts.

After the chromatin thread has become double, besides being thicker than before, it also possesses fewer coils, which is probably due to a longitudinal contraction of the whole spirem. At about the time when the nuclear membrane disappears the thickened chromatin band segments into the individual chromosomes

[13] Beiträge zur Kenntniss der Kerntheilung in den Pollenmutterzellen einiger Dikotylen und Monokotylen. Pringsh. Jahrb. f. wiss. bot. **30**: 175. 1897.

(*fig. 12*). The successive stages in the process of segmentation could be easily traced. The constrictions gradually become deeper and deeper, while at the same time the chromatin is withdrawn from the constricted regions. The resulting segments were always of the same length as the mature chromosomes appearing on the nuclear plate. Repeated examination of these stages failed to reveal a V-shaped bending back of the segments corresponding to that described by many investigators for Lilium. Moreover, the chromosomes do not have four lobes at the end, as would be more likely the case if they were formed by the folding-back process, but only two. It seems, therefore, that in Convallaria at least the chromosomes, although double in nature and hence sometimes showing a longitudinal split, are always straight and formed simply by the transverse fission of the chromatin thread.

The changes which the chromosomes pass through while on the nuclear plate are very difficult to make out, and consequently little that is definite can be said about them. The segments seem to be straight or slightly curved and lie mostly in a radial manner on the plate (*figs. 14, 19, 20*). The earlier stages show a nearly cylindrical chromosome, but very soon this becomes changed into the characteristic +-like structures which are very commonly seen at this stage. These structures seem to be formed, as Belajeff[14] and Strasburger[15] have shown, by a simultaneous splitting at each end of the cylinder, but in perpendicular planes. The inner forks are drawn apart by the spindle fibers, while the two outer ones separate in the plane of the nuclear plate. In many cases a fissure may be seen to extend from the apex of one long arm directly through the middle of the chromosome (*fig. 20*).

The two V-shaped segments resulting from the separation of the two halves of the + pass to the poles in the ordinary manner (*figs. 14, 15, 22*). The daughter segments proceed with the

[14] Zur Kenntniss der Karyokinese bei den Pflanzen. Flora, Erganzungsb. **79**: 434. 1894.

[15] Karyokinetische Probleme. Pringsh. Jahrb. f. wiss. Bot. **28**: 183. 1895.

angle of the V in front, as one might expect if the spindle fibers are assumed to be exerting a pull upon them. They are always more slender than the parent chromosomes, which would be the case if a division of the original substance had taken place. Occasionally a V will straighten out and lie along the spindle fiber, reaching almost from the equator to the pole.

The spindle in Convallaria is formed in the same way as in Lilium. The disappearance of the membrane is exactly coincident with the appearance of the kinoplasmic threads which immediately penetrate the nuclear cavity, and also extend outward into the cytoplasm (*fig.* 12). Very little light, however, could be thrown on the fate of the nucleolus, and its disappearance was very sudden. The multipolar spindle is not very distinct in this plant (*fig.* 13), and the poles scarcely ever extend much beyond the limits of the old nuclear membrane, and are often difficult to distinguish at all. The bipolar spindle is usually truncate at the poles, but unlike that of Potamogeton, it is broad and barrel-shaped, possibly due to the large number of chromosomes (*fig.* 14). In this respect it is similar to Lilium. In no case was there even so much as a granule present at the pole, or any thing that could be mistaken for a centrosphere. A strong nuclear plate follows the division, resulting in a cross wall separating the cell into two hemispherical parts.

SECOND NUCLEAR DIVISION OF THE MOTHER-CELL.

The resting stage between the first and second divisions in Convallaria is very short. One may often find the nuclei at one end of an anther in the cell-plate stage, while those at the other end have formed the nuclear plate for the second division. The daughter nuclei do not seem to pass entirely into a normal resting condition. So far as could be determined, no nucleolus ever appears, nor does the nuclear membrane become well developed, however a very delicate membrane may often be observed. The chromosomes apparently retain their identity throughout this stage. From the pole view it can be determined that they still possess their V-shaped form (*fig.* 16).

The transition from the resting stage to the second spindle is very abrupt. Two poles are formed in the cytoplasm, which move farther apart, so that a bipolar spindle is very quickly formed (*fig. 17*). In the preparations examined, no polar radiations or multipolar spindles were found. The already distinct chromosomes merely move together toward the center in order to form the nuclear plate. Even now most of them retain the V form, and only an occasional one becomes nearly straight. They are very irregularly arranged, so that the long arms of some project outward toward the poles, giving a ragged appearance to the plate, thus distinguishing it immediately from the plate formed in the heterotypic division.

It was found impossible to determine absolutely the nature of the segmentation, but from a study of all the stages obtainable it seems probable that the V is divided transversely. This of course would not mean a transverse division of the original chromosome, if the processes described for the first division are the true ones, but rather the completion of a second longitudinal splitting. No figures were found in any of the spindles that could be interpreted as a case of undoubted longitudinal splitting of the V after coming on to the nuclear plate of the second spindle. On the other hand, the nearly straight segments moving along the spindle toward the poles are all much shorter than the Vs, but of approximately the same diameter instead of narrower, as one would expect if longitudinal splitting had taken place.

The later stages are all perfectly normal. The chromosomes arrange themselves in the daughter nuclei and appear at length to fuse into a continuous chromatin thread. The nuclear membrane does not appear at once, but by the time the young pollen grains are differentiated it is usually evident.

The spindle during the second division, both before and after the passage of the segments to the poles, is much less distinct than during the first division, and it is composed of fewer fibers. After the daughter nuclei are formed, a cell-plate is deposited in the usual manner. The spindle now disappears and the tetrad division of the pollen-mother-cell nucleus is complete.

The number of chromosomes in Convallaria is quite large. A count in the nuclear plate stage showed eighteen segments as the reduced number. The same number may be counted during the subsequent resting stage and also after the second division.

THE MICROSPORES.

After the second division of the mother-cell nucleus the young pollen grains do not separate immediately, but remain a short time inclosed in the thickened walls of the parent cells. With little difficulty one can follow all the steps in the process of dissolution which these walls undergo. First the increasing sponginess of the already thick wall; a simultaneous differentiation of its inner layer destined to become the wall of the spore; and finally the complete solution of the outer part, leaving the young pollen grains united only by the intervening walls. These apparently split at once into two layers, thus freeing the members of the tetrad.

The pollen grains at first are quite small, and possess thin purple-staining walls, finely granular cytoplasm similar in consistency to that of the somatic cells, and a highly chromatic nucleus which occupies about one fourth of the cell-cavity (*fig. 23*). The further changes are mostly normal. The pollen grains, which from the first are elliptical, gradually increase in size until their volume is more than doubled. The wall increases in thickness, and the whole grain assumes a bluer tinge with gentian-violet.

A short time before the flower opens, the nucleus undergoes division, whereby a generative cell is cut off (*fig. 24*). This cell is lenticular in form, and separated from the general cavity of the grain by a distinct cell wall. The generative nucleus is exceedingly chromatic, so much so in fact that it stains almost a homogeneous dark purple with the gentian-violet. The generative cell in Convallaria seems to differ from those in most of the monocotyledons described by other writers in not separating at an early period from the wall of the pollen-grain. It apparently remains in all cases attached until the time of pollination.

The division of the generative nucleus into the two sperm nuclei must take place in the pollen tube, good stages of which were not obtained. Except in the one point above mentioned, the microspores of Convallaria do not differ in any essential way from those described by Strasburger[16] for a large number of the higher monocotyledons.

POTAMOGETON FOLIOSUS RAF.

The numerous investigations recently made upon plants belonging to the orders Alismaceæ and Naidaceæ have shown that many peculiar conditions are to be found among these groups of monocotyledons. During the past summer the writer was able to procure excellent material of *Potamogeton foliosus* in the ponds about Ithaca; and it was decided to make a detailed study of this plant for comparison with the studies already made by others. No one seems to have investigated this genus from a cytological standpoint.

Of the papers on nearly related plants must be mentioned that on Naias by Magnus,[17] on Naias and Zannichellia by Campbell,[18] and on Alisma[19] and Sagittaria[20] by Schaffner.

The material was collected during the months of July and August, at which time the oldest flowers are just producing fruit. The floral spikes mature in succession as the plant branches, so that the very youngest flowers and also the fruits may be found upon the same individual. There being no cutinized layer surrounding the bud, the latter is especially easy to penetrate with the fixing agent. The material used for this study was therefore in exceptionally good condition. As in the case of Convallaria, the collections were made at certain times during the day and night, and the result also was exactly the same.

[16] Befruchtungsvorgänge bei den Phanerogamen 22. 1884.

[17] Beiträge zur Kenntniss der Gattung Naias. Berlin. 1870.

[18] A morphological study of Naias and Zannichellia. Proc. Calif. Acad. Sci. III. 1:1. 1897.

[19] The embryo sac of *Alisma plantago*. BOT. GAZ. 21:123. 1896.

[20] A contribution to the life history of *Sagittaria variabilis*. BOT. GAZ. 23:252. 1897.

THE DEVELOPMENT OF THE MICROSPORANGIUM.

To trace the development of the anther, and especially the differentiation of the microsporangial archesporium in Potamogeton, is no less difficult than in Convallaria. Oddly enough, the only monocotyledonous type studied by Warming was one of the Naiadaceæ, namely Zannichellia. Warming thought that in this plant the process was essentially identical with that in the dicotyledons, but these results may be questioned, owing to the apparent paucity of material at his command.

The present study of Potamogeton seems to throw a little more light on the problem. In the young anther, which at maturity is always two-celled, there is found at each of the two more prominent angles of the quadrangular cross-section a single hypodermal cell, which at this stage is slightly larger than the surrounding cells and richer in protoplasm. This presently is divided by periclinal walls into two, and later into three daughter cells, each produced probably in centrifugal succession (*fig. 25*). The innermost of this series now immediately begins to enlarge, and becomes at once the primary archesporial cell. This cell undergoes rapid division, resulting at length in a number of cells, all formed from this one original archesporial cell. The irregularity in arrangement, and the gradual decrease in size from the center toward all sides, nevertheless suggest that some may owe their origin to the division of the surrounding tissue. The same gradual decrease in size takes place also on the side of the archesporium toward the connective.

It will be seen from the above account that the tapetum here and in Convallaria is not a morphologically distinct structure until at a comparatively late period in the development of the anther. It is not until the stamen is half mature that the archesporium becomes distinct from the wall. It can always be recognized at this period by the finely granular contents of the cells, just as was the case in Convallaria. No tapetum can be distinguished for some time. The cells of the inner layer of the wall, which from the first are smaller than the central cells, gradually take on a dense and partially disorganized appearance. There is

no longer any doubt but that the tapetum is differentiated from the wall, rather than from the archesporium, as a hasty inspection would seem to indicate.

The wall of the anther at this stage is composed of three, or rarely four, layers of cells (*fig. 26*). The outermost of these layers remains almost unchanged until the anther is mature, and is indeed the true epidermis. The other two or three layers have some of their cells arranged in more or less distinct radial rows, suggesting, as in the first case, that each row is the derivative of one hypodermal cell. The greater portion of the wall, however, is formed, as in Convallaria, from the cells of either side of the archesporium, and these are not necessarily derivatives of a hypodermal cell. Indeed, so far as could be determined, the growth was brought about exactly as in Convallaria. During the maturation of the anther the behavior of the cells is normal. The third layer undergoes disintegration, as does also the fourth, which is the tapetum. The epidermis remains normal, while at the same time the second layer becomes thicker walled than the rest, acquires spiral or reticulated thickenings, and is indeed a true endothecial layer (*fig. 27*).

Campbell considers the anther of Naias to be a so-called "caulome" structure, in which are early differentiated plerome and periblem, the upper cell of the plerome cylinder becoming the archesporium. This in itself does not preclude a process similar to that described above for Potamogeton; although Campbell himself is quite certain that the origin of the archesporium in Naias is not traceable to a single cell. He found the wall composed of only two layers besides the epidermis, instead of three. In Zannichellia the same difficulty was found in tracing the development, but this was probably because the tapetum was not counted as a wall layer. The archesporial cells are here also at first scarcely distinguishable from the adjacent cells. In this plant there are three layers surrounding the archesporium, all of which finally become disintegrated.

The complete disintegration of the tapetal cells in Potamogeton is almost coincident with the divisions of the pollen-mother-cell.

When the young pollen grains are free in the anther, therefore, only a disorganized mass of protoplasm is in the position formerly occupied by the tapetum. This substance is very soon distributed among the pollen grains, where it possibly serves as nutriment. The tapetal cells of Potamogeton never contain two nuclei. In this respect, therefore, they differ decidedly from Convallaria.

THE ARCHESPORIUM AND MOTHER-CELLS.

Division in the primitive archesporium ceases at an early period, after which the development is confined to growth and constitutional changes in the cells already formed. The definitive archesporial cells are at first quite small, but during the long period of growth that now commences they double or even triple their original size. The mature pollen-mother-cell contains a very large nucleus surrounded by abundant cytoplasm. Unlike most monocotyledons, the cell wall here remains very thin, and does not become irregularly thickened, as in Convallaria and Lilium (*fig. 35*). A similar condition has been observed also in Naias and Zannichellia. A very short time, therefore, is required for disintegration, which undoubtedly accounts for the almost immediate separation of the young pollen grains.

THE ARCHESPORIAL NUCLEUS.

Potamogeton belongs to an entirely different class from Convallaria so far as the nuclei are concerned. The Lilium type, to which the latter plant belongs, possesses the well-known dense spirem and the large oblong chromosomes. The nuclei of Potamogeton are apparently very poor in chromatin. The few chromosomes are small and spherical and the spirem very meager. A detailed comparison with Convallaria, therefore, will be especially interesting.

The very young archesporial nuclei in Potamogeton are scarcely different from the surrounding vegetative nuclei. They are surrounded by a definite membrane, have a large nucleolar-like body, and a very poor linin network, which lies close to the

wall (*fig. 28*). As the cell gradually expands, the nucleus also increases in size. The nucleus just before synapsis has already acquired nearly its full size, and the linin network composed of very slender threads is plainly visible. In it are irregularly distributed a few large and small granules of chromatin. Both the linin and the granules are exceedingly meager as compared with Convallaria. The nucleolus is a gigantic body, much larger than those in most plants, and takes the gentian-violet stain readily, making it thus a very striking object in the cell. Attached to it on one side is a small wart-like body only slightly larger than the largest chromatin granules. Rarely two of these are present. This body appears very much like a bud produced by the nucleolus itself, but in reality is not. If specimens are examined which have been poorly stained, in which the gentian-violet has been mostly washed out, this little body remains dark much longer than the nucleolus. A fine double stain can often be thus obtained. The nucleolus takes the orange in that case, while the other body stains violet. From the fact that this larger body always stains like ordinary chromatin, and since there is scarcely any chromatin upon the linin thread, the writer is inclined to believe that it is not the nucleolus, but a large mass of chromatin similar to those found in many animal cells. The nucleolus is possibly the wart-like body attached at its side. The behavior of the larger body when the chromosomes are being formed seems also to support this view.

The synapsis stage in Potamogeton is even more marked than in Convallaria (*fig. 29*). The linin network contracts into a globular mass lying in contact with the nuclear wall, but not noticeably pressed against it. With the highest magnification, the central part of the mass still appears too dense to distinguish any structural characters. On the periphery, however, the free ends of the network may be seen easily. The large nucleolus, accompanied by the wart-like body, remains in its central position in the nucleus throughout the synapsis stage. It always stains more deeply than the linin. The mass of linin, therefore, lies between the nucleolus and the nuclear membrane. The later

stages of synapsis are marked by the same striking peculiarities that were met with in Convallaria (*fig. 30*). In just the same way large globules of some deeply stainable matter accumulate on the outside of the contracted mass, appearing as if expelled from it. They seem, however, to be distinct from the nucleolus, as there is no apparent fragmentation nor budding of the latter. The nucleolus throughout the whole process remains of exactly the same size and regular contour, and with the little wart-like attachment undisturbed.

The spirem stage is much shorter than in Convallaria. The contracted linin network gradually begins to expand until the threads are again spread out beneath the membrane. Here again we notice a decided change in the structure of the linin thread, just as was the case in Convallaria. It is no longer so slender, and provided with such large granules, nor is it so conspicuously in the form of a network. The spirem is composed of a few rather thick linin threads extending in various directions around the nucleolus, and crossing each other occasionally. In them appear small chromatin granules, which, however, are much smaller and more regular in size than those present before synapsis (*fig. 31*). The whole process, therefore, is exactly comparable with that in Convallaria.

FIRST NUCLEAR DIVISION OF THE MOTHER-CELL.

The stages in the nuclear development preparatory to the first nuclear division are not nearly so marked as in Convallaria. The first indication of division is found in the gradual massing together of the chromatin into a number of irregular masses simulating those found just before synapsis. The difference lies in their larger and more equal size. Just at the time when the nuclear membrane is disappearing the number of these masses of chromatin may be determined approximately. Probably fourteen or sixteen is the correct number. They soon seem to lie together in pairs, in which case two may be easily mistaken for one (*fig. 32*). During the later stages the two parts of each pair seem to lose their identity, so that when on the spindle

it is not possible to count more than seven or eight chromosomes.

Whether a fusion takes place here one cannot determine, since the small size renders it impossible to follow the process accurately. The exact manner of segmentation upon the spindle is also still in doubt. Seven chromosomes are found in the daughter nuclei, which leads us to infer that each of the original masses splits into two, but the preparations show no indications of any such division farther than that in many cases under high magnification, it seemed as if the chromosomes possessed a +-like structure similar to that in Convallaria, but the figures were not distinct enough to allow of any definite conclusions.

In regard to the formation of the spindle a few notes may be given, although the results do not differ essentially from those obtained by Mottier in Lilium. The kinoplasm is at first limited to a thin felt-like coat surrounding the nucleus (*fig. 32*). On account of the very large space occupied by the nuclear sap, it is easy to observe the entrance of the kinoplasm into the nuclear cavity. This takes place apparently before the entire disappearance of the membrane. The latter sometimes is still visible after the nuclear cavity is nearly filled with kinoplasm. At first thought it seems impossible to conceive of a substance passing through the nuclear membrane in this way. But Mottier[21] has shown that the membrane itself is probably nothing more than a close weft of kinoplasm. We have then merely to assume that the inner threads of this weft separate from the rest and traverse the nuclear cavity instead. Finally the whole membrane is entirely transformed into radiating threads. Thus it is not necessary to conceive of the kinoplasmic threads penetrating the membrane. They are from the first a part of it. At first the spindle is multipolar (*figs. 33, 34*), but the poles are few in number and very soon disappear, thus giving place to the normal bipolar type. In its mature condition the spindle is narrow and the poles are very acute (*fig. 35*). The fibers

[21] *Op. cit.* Pringsh. Jahrb. f. wiss. bot. **30**: 176. 1897.

are few in number, probably not exceeding the number of chromosomes.

In many cases the point at the pole toward which the spindle fibers converge was occupied by a granule both in the first and second division spindles. This granule in well-stained preparations was always dark, but its inconstant occurrence was decidedly against its being considered a permanent structure. The cell plate forms before the spindles of the second division (*fig. 36*).

The body, which looks so much like a nucleolus, disappears previous to the first division at almost the same time as does the nucleolar membrane. At this period it presents a more or less irregular and lobed appearance, but vanishes so quickly that it was impossible to determine whether the process was one of fragmentation or solution.

SECOND NUCLEAR DIVISION OF THE MOTHER-CELL.

Before the second division there is a distinct resting stage. An indistinct membrane is formed, and even a nucleolar body may appear. This latter, however, never becomes so large as in the archesporial nucleus, and often seems to be entirely absent, or at least indistinguishable from the chromosomes (*fig. 36*). A thick linin thread is usually formed, but the chromosomes remain distinct. In this character Potomageton agrees well with Convallaria. During this resting stage it is again possible to count the chromosomes, when the number is still found to be seven or eight. This resting nucleus can be distinguished easily by its much larger size from the one formed after the second division.

The origin of the spindle could not be traced, but many preparations showed it in the mature condition (*fig. 37*). These spindles are smaller and more slender than those described above. Like the latter, they have very pointed poles, and in both cases the poles are almost if not quite in contact with the cell wall.

The chromosomes are closely aggregated in the nuclear

plate stage, so that it was impossible to determine just what happened to them at this point. The daughter chromosomes move to the poles very evenly, that is, with the same degree of rapidity (*fig. 38*). A count here showed the seven segments present. Although the daughter nuclei are quite small, the chromosomes remain distinct for some time, and can be again counted. After a time the nucleolar bodies also reappear, and the division is then complete.

THE MICROSPORE.

Our knowledge of the internal structure of the pollen grain really dates from the time of Hartig.[22] In this author's work is the first mention of the discovery in Tradescantia and several other plants of two nuclei in the pollen. This important discovery seems not to have been noticed by subsequent investigators until Strasburger's exhaustive work appeared in 1877.[23] It was not until then that the fact was generally recognized that two nuclei are to be found sooner or later in the development of every angiospermous microspore. This author was also able to demonstrate that the larger of these two nuclei is to be considered as a vegetative or prothallial nucleus and the smaller a generative nucleus.[24] Strasburger found also that a second division takes place regularly in angiosperms, either in the spore itself or in the pollen tube just before fertilization. Two sperm cells are thus formed from the one generative cell.

Since that time many investigators working upon widely different plants have found two nuclei, and these observations all lead to one result, namely the confirmation of Strasburger's observations in every essential particular. The time of formation, ultimate shape of the generative cell, and the time when the latter divides were indeed not always the same in different plants; on the contrary, all degrees of variation were found, some of which are noted below.

[22] Botanische Untersuchungen aus der physiologische Lab. Land. Lehrung. Berlin, herausg. Karsten **3**: 294. 1866.

[23] Befruchtung und Zelltheilung 18, 1877.

[24] Befruchtungs Vorgänge bei den Phanerogamen 5, 1884.

The microspores of Potamogeton become separate immediately after the second division of the mother-cell. The anthers are at this time still quite small, the subsequent growth being in reality for the purpose of accommodating the increase in size of the pollen grains. The microspores at first have a thin, although distinct and homogeneous cell wall surrounding the cytoplasm, and a very large nucleus. The latter fills at least one fourth of the whole cavity of the cell (*fig. 40*). The limited amount of cytoplasm present at this time is decidedly much more homogeneous than in the mature pollen, and stains with the gentian-violet a uniform pale violet similar to that of the cell wall. For a short time after the wall of the mother-cell disintegrates the pollen grains are still held together by the remains of these walls. In fact they are as if imbedded in a ground mass of some viscid matter.

The young grains very soon begin to increase in size, but the cytoplasm does not keep pace. As a result, the latter at length is confined to the parietal layer, but with a considerable increase in thickness on the side where the nucleus is located. These stages occur when the embryo-sac is yet one-celled, and of course while the spike of flowers is still enclosed within the bud.

Just before the nucleus begins to prepare for division we find the following conditions: The cytoplasm is decidedly more granular, and stains more deeply with the orange. The large vesicular nucleus possesses a very distinct membrane. Lying close against this is the linin thread which is rather extensive for Potamogeton. The thread however is nearly destitute of chromatin. The nucleolar-like body is smaller than usual, and in some cases more than one may occur.

The first division of the microsporial nucleus takes place much earlier than in Convallaria, and while the whole spike is yet enclosed in the bud. The spores reach their full size before the division, and it is at this time that the exine first begins to show signs of the thickening which produces the very slightly roughened surface of the mature spore. Owing to the small

number of chromosomes present, the spindles in the pollen grain are exceedingly minute and slender (*fig. 41*). The process of division here does not seem to present any new features. When preparing for division, the primary nucleus moves toward one side of the cell, so that the resulting spindle has one pole in contact with the cell wall. This pole unlike the free one, is not pointed; on the contrary, it is usually quite broad, so that the spindle fibers are attached to the wall over a considerable area. The spindle is quite dense and stains readily, but is composed of few fibers. After the chromosomes pass to the poles a distinct cell-plate forms, and is later followed by a definite membrane (*fig. 42*). The latter is arched in such a way as to cut off one daughter nucleus in a small lenticular cell, of which one wall is the wall of the spore itself. This is the so-called generative cell.

The cytoplasm henceforth occupies the greater part of the cell cavity. It gradually becomes filled with large bodies which stain purple with gentian-violet, and blue with iodine. They are in reality starch grains. A similar occurrence of starch in the pollen grain has been described in Naias by Campbell.[25]

After a short period of rest the generative cell begins to elongate, notwithstanding the fact that it still appears to be closely attached to the wall. The elongation produces an oblong cell, and is the first step in preparation for the second division. The chromosomes for this division are formed early. They can often be seen to occupy nearly the entire nuclear cavity, and so distinct are they in many cases that one may count them. The number here again is uniformly seven. The spindles were found in considerable numbers, one of which is seen in *fig. 43*. The spindle fibers are very coarse and apparently scarcely more numerous than the chromosomes. They seem to stain more easily than is ordinarily the case in this plant. The chromosomes are during this division exceedingly minute.

[25] Proc. Calif. Acad. Sci. III. 1:16. 1897.

The cell plate is soon deposited, and divides the generative cell into two parts. The two daughter cells do not separate, but remain connected as a two-celled body during their entire stay in the spore. A more or less prominent constriction often occurs at the middle, but this does not seem to be constant (*fig. 44*). The pollen grain is always somewhat flattened, and since the generative nucleus is usually adjacent to the flat side, it is not possible to tell whether the latter remains attached to the wall after division. The difficulty was increased since the pollen escapes from the anther very soon after the second division.

An examination of the literature relating to the pollen grain of the monocotyledons furnishes some interesting facts. Strasburger found the division of the generative cell to take place within the spore only in Juncus and Arum; while in all other cases the division was in the tube.[26] All these cases belong either to the Liliaceæ, Orchidaceæ, Amaryllidaceæ, or Iridaceæ. Schaffner found the division occurring in the spores of Typha, Alisma, and Sagittaria,[27] and Campbell found the same to be the case in Naias, while the writer finds the same phenomena in Acorus and Potamogeton. In all cases among the monocotyledons, where division occurs in the pollen grain, with the exception of Alisma and Sagittaria, the generative cell is at first enclosed by a wall, and always becomes two-celled after division, although Campbell claims that the two cells in Naias separate before passing into the tube. Schaffner was not able to discover any walls around the generative cell in the two above mentioned species. From this it appears that the division of the generative nucleus in the tube is mostly confined to the liliaceous and orchidaceous groups among the monocotyledons, while the division within the spore characterizes the spadiceous and naiadaceous groups.

[26] Befruchtungsvorgänge bei den Phanerogamen 22. 1884.

[27] A contribution to the life history of *Sagittaria variabilis*. BOT. GAZ. **23**: 252. 1897.

SUMMARY.

The following brief summary may aid in bringing together the results reached in the foregoing pages.

The experiments with regard to the effect of external conditions on nuclear division both in Convallaria and Potamogeton gave no results for light and humidity, which were the only conditions tested.

The material illustrating the younger stages in the development of the microsporangium shows that the process is slightly different in Convallaria and Potamogeton from the normal method as given by Warming and Engler. The archesporial cells arise by the division of a hypodermal cell at one corner of the anther. Therefore, instead of the archesporium arising from a layer of hypodermal cells, as Warming describes for dicotyledons, it arises from one or rarely two hypodermal cells. The primary archesporial cells divide only a few times, but there is considerable subsequent growth in size of each cell. The next outer cell in the original row forms part of the tapetum, and the remainder are wall cells. Most of the wall and tapetum, however, is formed from the tissue at either side of the archesporium and in its rear. This differs from Warming's views mainly in the restriction of the hypodermal cell and in the derivation of the wall from the adjacent tissue. The anthers of all other monocotyledons which the writer has had an opportunity to examine seem to show that this is more likely the normal process for the whole group.

The tapetal nuclei of Convallaria show nicely the process of nuclear fusion which has been described by Strasburger and others for many other plants. After the division of the primary tapetal nucleus by the mitotic method, the two daughter nuclei in many cases fuse again, and all stages of the process may be found often in the same anther. It is probable that not all the nuclei divide, and also that not all of those that do divide fuse again before disintegration. It seems that in Potamogeton no division of the tapetal nuclei takes place.

The structure of the wall in the mature microsporangium

was found to agree with the monocotyledonous type in general. The sequence in development was centrifugal and resulted in a well-defined endothecial layer, together with two or three inner wall layers in addition to the epidermis. At maturity only the epidermis and endothecial layer are present.

The development of the archesporial nucleus shows some very important features. The contracted condition called synapsis is without doubt a normal process accompanied by radical changes in the chromatin thread. The latter, which before synapsis was in the form of a network in which were imbedded large irregular chromatin masses, after synapsis is thicker, coil-like, and with the chromatin in smaller more equal masses. The spirem therefore begins at the close of the synapsis stage. In both plants studied irregular dark masses were apparently expelled from the chromatin thread at the close of the synapsis, and in Potamogeton at least it was plainly evident that these had no connection whatever with the nucleolus. The ultimate fate of this chromatin-like matter was not determined. Whether this phenomenon was artificial or natural could not be determined from the material at hand.

The growth and segmentation of the spirem in Convallaria is almost identical with that in Lilium as described by Mottier. The longitudinal splitting of the ribbon is especially noticeable. In Potamogeton the process is different but could not be worked out satisfactorily owing to the minute size of the nuclei. In this plant sixteen chromatin masses were counted just before division, but later there were only about seven, seeming to indicate a fusion of the widely separated primary segments to form the chromosomes. The number of chromosomes after reduction was eighteen in Convallaria and seven in Potamogeton. This last number is one of the smallest so far recorded for the phanerogams.

Spindle formation in all three plants agrees in every essential particular with the process described by Strasburger and Mottier for Lilium. The multipolar condition was evident in each case, but was less distinct in Convallaria.

The splitting of the chromosomes in the heterotypic division

was in Convallaria exactly similar to that in Lilium. All the stages were especially clear. The + formation, however, began in some cases during the early multipolar condition. The process in Potamogeton was probably also normal.

Nothing new could be determined in regard to the segmentation during the second division. It seemed to be absolutely impossible to determine in these plants with any degree of certainty whether the division was transverse or longitudinal. All the phenomena, however, seemed to indicate a transverse rather than a longitudinal division in both plants.

The walls of the mother-cells in Potamogeton were thin as in Naias and Zannichellia.

In the microspores of Convallaria the generative nucleus is very chromatic, and is cut off by a distinct wall, but does not become detached from the wall of the spore until just previous to the time of passing into the tube. The division of the nucleus is probably in the tube since it was not found within the spore. As in Convallaria, so also in Potamogeton the generative cell is cut off very early, but in the latter plant the two sperm cells are immediately formed. The two male nuclei are inclosed each within its own cell wall, but they both still remain attached to the wall of the spore. The two-celled body then passes down the tube and even into the egg without separation of the two cells. The spindles in each case are very small and the chromosomes very minute.

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EXPLANATION OF PLATES XXIV–XXV.

PLATE XXIV. Convallaria majalis L.

FIG. 1. One of the angles of a young anther in cross-section, showing the row of cells derived from the primary hypodermal cell; the two inner are doubtless archesporial cells; the third will form part of the tapetum.

FIG. 2. An anther cell at a later stage; the two archesporial cells in *fig. 1* have divided several times forming a small mass of tissue; the row of wall cells at the top is probably equivalent to that in *fig. 1*; the tapetum is not yet formed.

FIG. 3. A section of the anther wall at a still later stage; on the extreme outside is the epidermis; then two layers the inner one of which will later disintegrate; the fourth layer is the tapetum the cells of which often contain two nuclei; and farther inside are shown a few archesporial cells.

FIG. 4. A portion of the anther wall at nearly the same stage as in *fig. 3*, showing the division of the tapetal nucleus.

FIG. 5. A slightly later stage of the same in which the daughter nuclei formed in *fig. 4* are in the process of fusion.

FIG. 6. A portion of the mature anther wall; the outer layer is the epidermis, the next below is the endothecium with spiral markings on the walls; and farther inside a disorganized mass composed of the tapetum and the one or two layers just outside.

FIG. 7. An archesporial nucleus in the resting stage; the linin network contains granules of chromatin of various sizes.

FIG. 8. Synapsis, the projection at the left is the nucleolus.

FIG. 9. Last stage of synapsis; the spirem ribbon is opening out, and between its meshes are chromatin masses of various sizes; the nucleolus at the left.

FIG. 10. The spirem ribbon with chromatin granules imbedded in the linin.

FIG. 11. The spirem ribbon after longitudinal segmentation, and cut in lengths by the section knife.

FIG. 12. Chromosomes still showing the double nature; dissolution of the nuclear membrane.

FIG. 13. The multipolar spindle with chromosomes in various views.

FIG. 14. The bipolar spindle and nuclear plate; the chromosomes from the end appear +-shaped, from the side view more elongated.

FIG. 15. The + separates into V-shaped segments which are seen moving toward the pole.

FIG. 16. During the resting stage before the second division, the nucleus viewed from the pole; the chromosomes remain distinct.

FIG. 17. The nuclear plate of the reducing division; the segments are so numerous that their form can be determined only with difficulty.

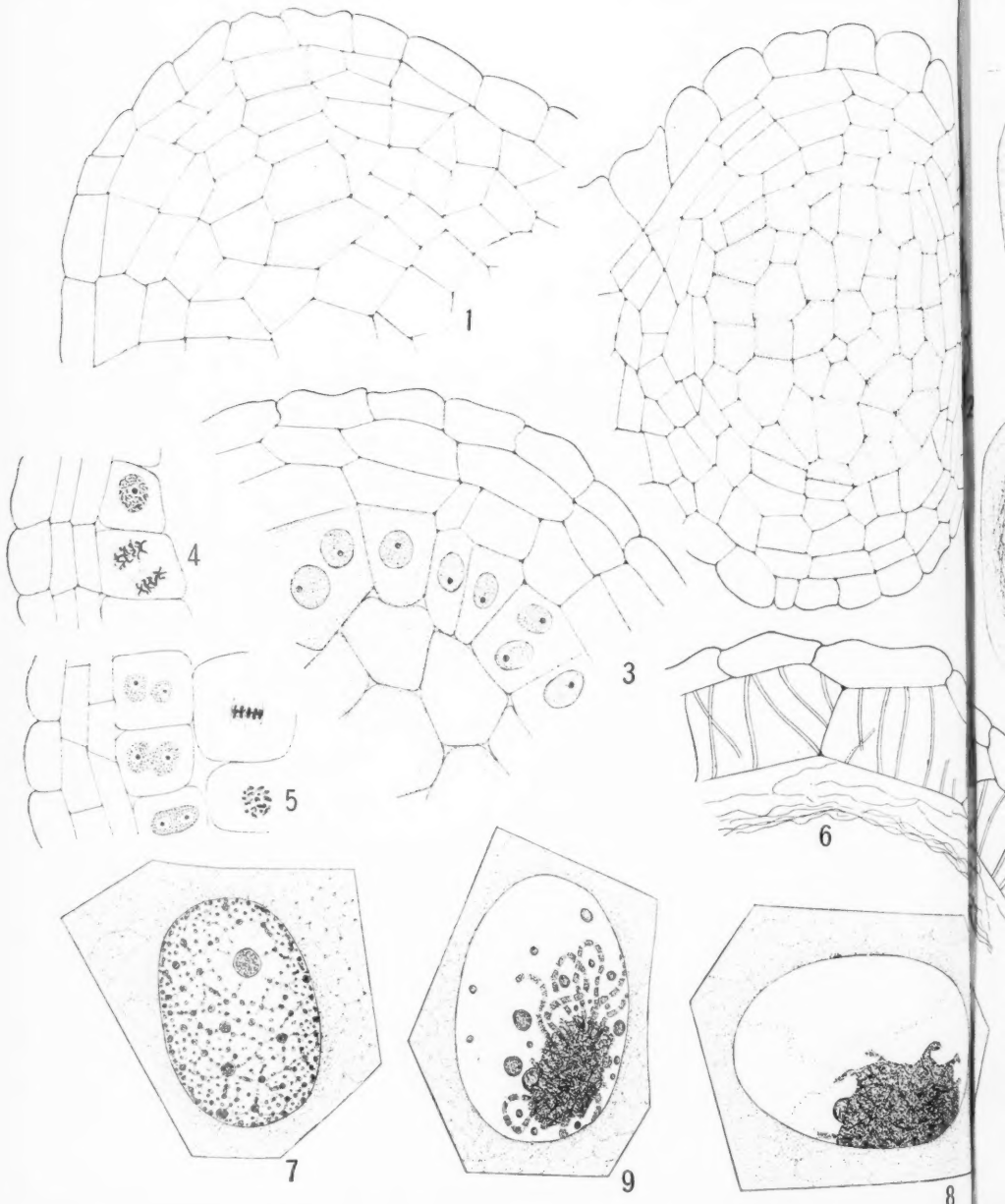
FIG. 18. A chromosome on the nuclear plate, side view.

FIG. 19. Pole view of a chromosome which is bent V-shaped.

FIG. 20. End view of *fig. 18*.

FIG. 21. A rare case, where *fig. 20* has opened out along the fissure line forming a ring.

FIG. 22. V-shaped segments ready to pass to the poles.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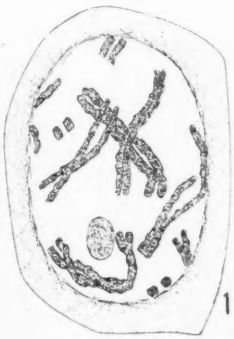
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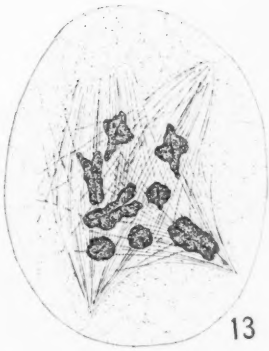
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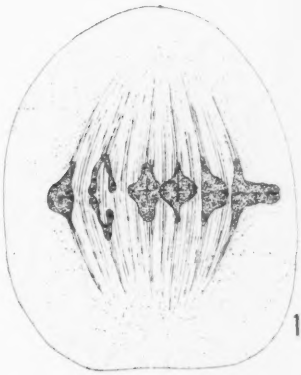
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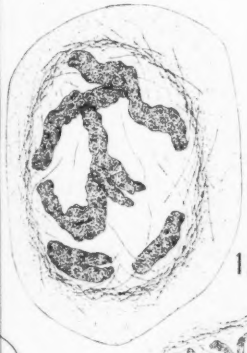
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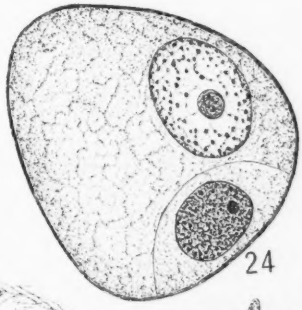
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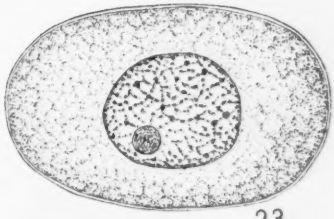
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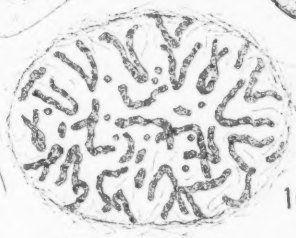
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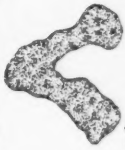
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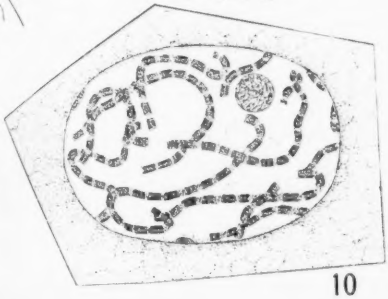
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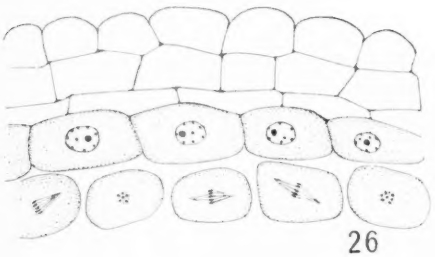

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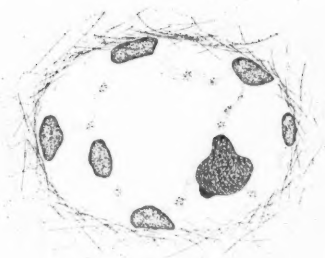

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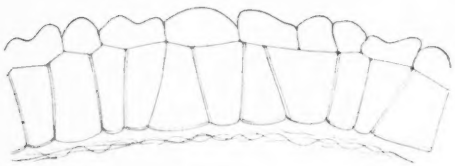

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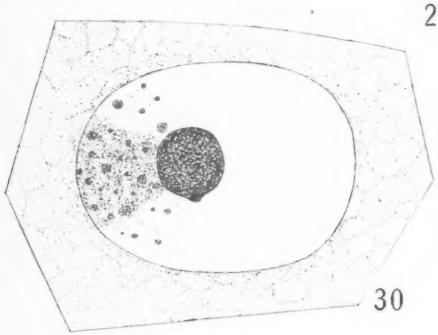

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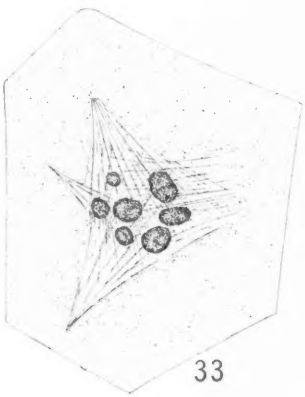

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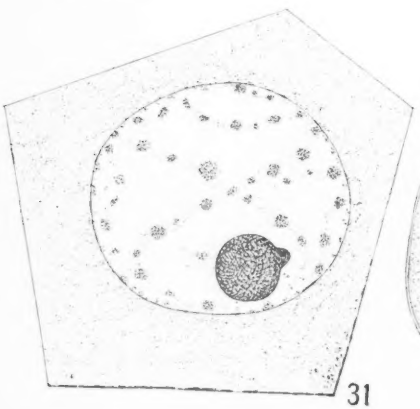

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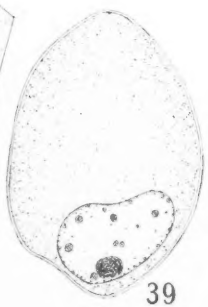

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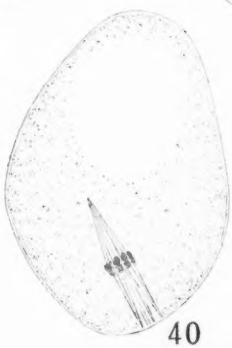

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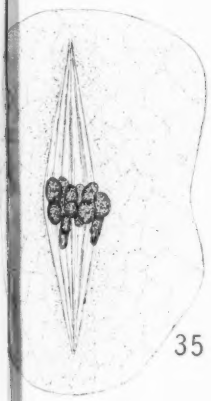

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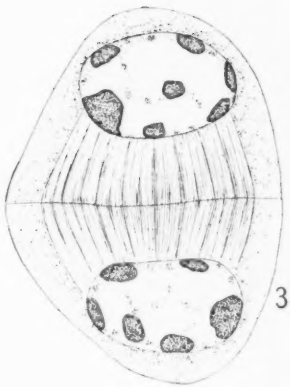

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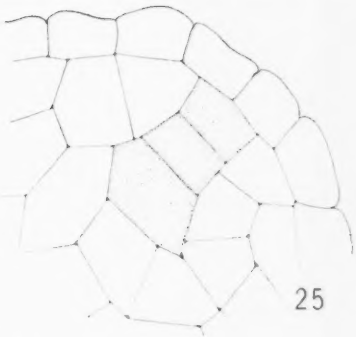

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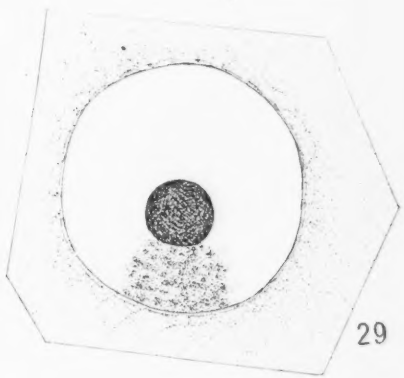

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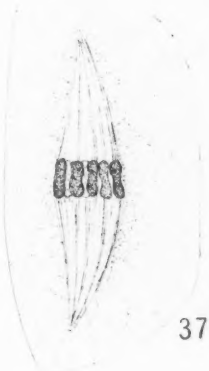

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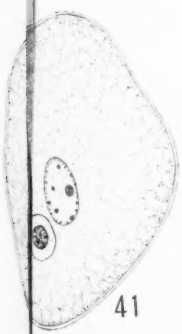

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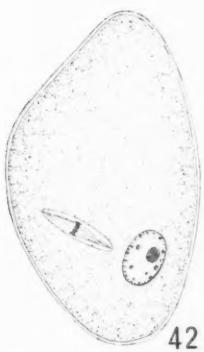

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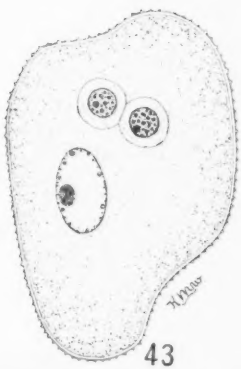

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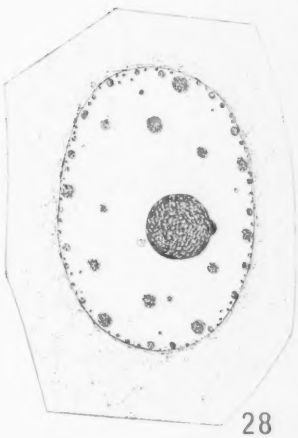

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FIG. 23. The microspore before the division of the primary nucleus.

FIG. 24. Same after the generative cell has been cut off; the generative nucleus is very dense.

PLATE XXV. Potamogeton foliosus Raf.

FIG. 25. The row of cells derived from the primary hypodermal cell at each corner of the young anther.

FIG. 26. A portion of the anther wall in a later stage; the outer layer is the epidermis, the second becomes the endothecium, the third disintegrates, the fourth is the tapetum, and farther inside are some archesporial cells.

FIG. 27. The structure of the mature anther wall; the endothecium with its indistinctly spiral markings is limited on the outside by the epidermis, and within by the disintegrated wall-cells and tapetum.

FIG. 28. An archesporial nucleus in the resting stage; the indistinct linin network contains a few pale-staining chromatin granules; the large dark mass is the nucleolar-like body; and the black bud at the side is possibly the nucleolus.

FIG. 29. Synapsis; there is very little chromatin in the contracted mass.

FIG. 30. Later stage of synapsis; granules are expelled and the linin is beginning to spread out.

FIG. 31. The spirem; contains very little true chromatin.

FIG. 32. The nucleolar body has disappeared and at the same time the chromosomes were formed; the membrane is here spreading out.

FIG. 33. The multipolar spindle and chromosomes.

FIG. 34. Same becoming bipolar.

FIG. 35. The bipolar spindle and nuclear plate; the chromosomes seem to be double.

FIG. 36. Daughter nuclei after the first division; the chromosomes remain distinct.

FIG. 37. The second or reducing division, nuclear plate stage.

FIG. 38. Same showing the daughter segments moving toward the poles.

FIG. 39. The reducing division; chromosomes in the nuclear plate.

FIG. 40. A microspore with its primary nucleus.

FIG. 41. Same showing the mitotic division of this nucleus.

FIG. 42. Same with a free vegetative nucleus, and a small dense generative nucleus inclosed by a convex wall.

FIG. 43. Same showing the division of the generative nucleus.

FIG. 44. A mature microspore in which the two dense sperm nuclei are each enclosed by a cell wall.

BRIEFER ARTICLES.

AN HERMAPHRODITE GAMETOPHORE IN PREISSIA COMMUTATA.[1]

(WITH ONE FIGURE)

THE reproductive organs of most of the Marchantiaceæ are borne on specialized receptacles known as gametophores. So far as I can learn there has been no instance recorded of the two sex organs being found on the same gametophore. In a recent study of Preissia, however, I discovered this phenomenon.

The normal archegoniophore of Preissia is hemispherical in shape, the archegonia being situated on the lower surface. The tissue adjacent to the archegonia is compact and is made up of small cells rich in protoplasm. The tissue in the upper part, however, is much looser and the cells are larger. The antheridiophore is discoid, with the upper surface slightly concave, in which the antheridia are sunk. The tissue, particularly that surrounding the antheridia, is much looser than that of the female receptacle, and the cells are somewhat larger.

The shape of the hermaphrodite gametophore from which the accompanying illustration was made clearly indicates that it is primarily an archegoniophore, but it is modified to adapt it to its peculiar conditions. The upper part is more strongly developed than normally, and is irregular in form. The archegoniophore at maturity has a long stalk, but as the material from which this section was made was put up in the fall the stalk had not yet elongated. On the under surface is shown a portion of one archegonium, the neck having been cut off in sectioning. In the upper portion of the gametophore are two well-developed antheridia, corresponding almost exactly in shape and size to those occurring in normal plants. Their structure is also the same. Their position in the gametophore is very similar to that which they

[1] Miss Townsend discovered this interesting case of an hermaphrodite gametophore while engaged in the regular course of advanced work in comparative morphology, and it was at my suggestion that she has prepared it for publication.— GEO. F. ATKINSON.

occupy in the normal antheridiophore. The tissue surrounding them resembles that of the latter receptacle, while the tissue of the lower portion is that of the ordinary archegoniophore.

There is a question whether this is merely an abnormal condition or whether it is a reversion to an earlier type. In the Ricciaceæ the two organs may either be produced together or, as is often the case with *Riccia glauca* and *Riccia hirta*, they may be found on the same part of

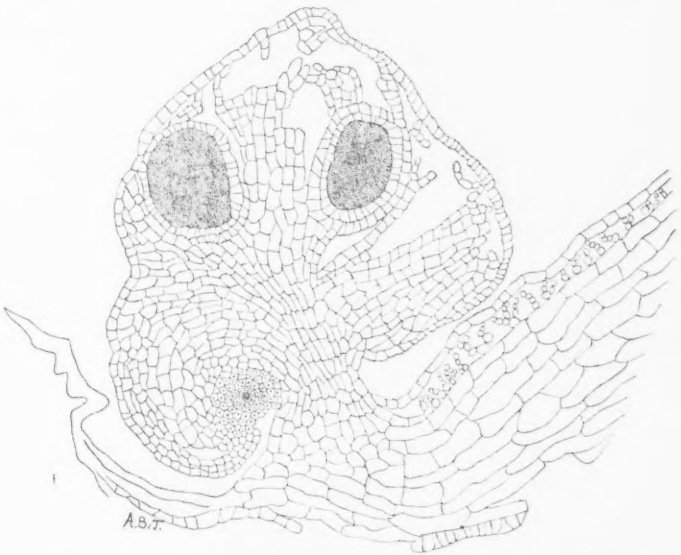

FIG. 1.—Section of hermaphrodite gametophore of *Preissia commutata*.

the thallus, but occupying different areas, one organ being produced for a time and then the other. It is possible that in the evolution of the gametophore the tissue surrounding the reproductive organs may have become differentiated to form a receptacle, and, as the two organs were situated near each other, they may at first have been borne on the same gametophore. As the reproductive organs became more highly developed and specialized, the gametophore might naturally have advanced in development and specialization, and in time have come to produce but one kind of organ. A still further development might then have produced two distinct gametophores, each adapted in form and structure

to the functions of the organ borne on it.* A suggestive case in the algæ occurs in *Vaucheria geminata* and *V. terrestris*, where an unspecialized branch, or gametophore, bears both antheridia and oogonia. It is possible that the original type of gametophore was hermaphrodite. In *Vaucheria terrestris* there are occasional branches which bear but one organ, showing that a unisexual gametophore might be developed from the hermaphroditic.—ANNE B. TOWNSEND, *Cornell University*.

SOME PLANTS RECENTLY INTRODUCED INTO FLORIDA.

TWO YEARS since[2] I called attention to some South American species found by Mr. A. H. Curtiss in ballast or about streets in various parts of Florida. A package of specimens recently sent for study by Mr. Curtiss contains several South American and southwestern species apparently not before reported from Florida, most of them unrecorded from the North American continent. The plants from Pensacola were collected, in the words of Mr. Curtiss, "from lands lying inside the wharves, such as presumably consist more or less of ballast earth." This fact suggests that *Jussiaea suffruticosa*, and *Hydrocotyle bonariensis*, formerly reported from Pensacola, are perhaps introductions to be classed with *Alternanthera pungens*, *Solanum elæagnifolium*, and *Anthemis mixta* (recorded in the article referred to), and that they have reached the Florida coast through the same channel as the following species:

IPOMŒA PALMATA Forsk. Fl. Ægypt. Arab. 43.—This Egyptian plant has been recently introduced into Florida where it has made itself at home, growing vigorously in waste ground about St. Augustine, Jacksonville, and Pensacola (no. 6496). At Pensacola Mr. Curtiss states "I found it growing rampant over bushes on the bay shore and fruiting freely."

SOLANUM GLAUCUM Dunal in DC. Prodr. **13**[1]: 100.—A Brazilian species which was first noted at Pensacola in 1897 when it was not abundant, though now thoroughly at home (no. 6530).

SALPICHROA RHOMBOIDEA Miers in Hook. Lond. Jour. Bot. **4**: 326. 1845.—This delicate solanaceous plant, native of Argentine Republic, was recently collected by Mr. Curtiss (Oct. 3, 1899) in low ground at Jacksonville (no. 6542).

[2] BOT. GAZ. **24**: 433–436. 1897.

ASTER DIVARICATUS Torr. & Gray, var. GRAMINIFOLIUS Baker in Martius, Fl. Bras. **6**[3]: 22.—This common South American form has been found for three years about wharves at Pensacola where it seems permanently established (no. 6497).

ERIGERON BONARIENSIS L. Sp. Pl. **2**: 863. 1753.—Growing luxuriantly (6 or 7 feet high) about the wharves at Pensacola (no. 6499). Introduced from South America.

PASCALIA GLAUCA Orteg. Hort. Matr. Dec. 39. This Chilian species has been introduced within a year or two at Pensacola (no. 6492).

PECTIS PROSTRATA Cav. Ic. **4**: 12. *pl. 324.* Very recently introduced at Pensacola (no. 6531): abundant from the southwestern states south through Mexico.—M. L. FERNALD, *Gray Herbarium.*

CURRENT LITERATURE.

MINOR NOTICES.

LIEFERUNGEN 190–192 of Engler and Prantl's *Pflanzenfamilien* have recently been published. They are devoted to a continuation of the Polypodiaceæ by L. Diels.—C. R. B.

THE PAPERS of botanical interest printed in abstract or in full in the *Proceedings* of the Indiana Academy of Science for 1898 (published 1899) are as the following. THOMAS: Some desmids of Crawfordsville; MOTTIER: Nuclear division in vegetative cells; The centrosome in cells of the gametophyte of Marchantia; Endosperm haustoria in *Lilium candidum*; RISLEY: Absorption of water by decorticated stems; ARTHUR: Indiana plant rusts, listed in accordance with latest nomenclature; SNYDER: The Uredineæ of Madison and Noble counties, with additional specimens from Tippecanoe county; GOLDEN: *Aspergillus oryzæ*; CURTISS: A red mold; OLIVE: Affinities of the Mycetozoa; CUNNINGHAM: Morphological characters of the scales of Cuscuta; COULTER; Notes on the germination and seedlings of certain native plants; BRANNON: Some Indiana mildews.—C. R. B.

STATISTICAL methods have come into greater prominence in biological study during recent years. Dr. Charles B. Davenport has prepared a useful little handbook,[1] in which, after some preliminary definitions, he sets forth the proper methods of measuring and counting organisms, of seriation and plotting of data, describes the constants of plotted curves and probable errors in their determination, and enumerates the classes of plotted curves. A chapter is devoted to correlated variability and the methods of determining the degree of correlation and heredity. Galton's, Pearson's and Duncker's methods of determining the coefficient of correlation are given. Formulas, rules, and ten tables useful for the various calculations make up the bulk of the handy volume.

A short chapter on the applications of statistical biological study calls for remark. That these methods are of great value for a study of variation and heredity admits of no doubt. That they will improve our conceptions of species and varieties is not at all clear. That "by the use of the quantitative method biology will pass from the field of the speculative sciences to that of the exact sciences" is surely a vain hope. We shall see the pendulum

[1] DAVENPORT, C. B.: Statistical methods with special reference to biological variation. 12mo. pp. viii+148, *figs. 28*. New York: John Wiley and Sons. 1899. $1.25.

swing far toward the side of quantitative study and then back to a point nearer equilibrium.—C. R. B.

THE DEPARTMENT OF AGRICULTURE issues as one of its bulletins the first part of a useful little book, by the Chief of the Division of Forestry under the title, *A primer of forestry*.[2] In four chapters Mr. Pinchot presents in simple language (1) an account of the structure and activities of a tree as an individual; (2) the relations of trees in a forest, including their requirements as to light and moisture, and their reproduction; (3) the life of a forest, discussing the origin of the forest, the struggle between the trees, their death, and the modes of lumbering; (4) the enemies of the forest, including man, grazing, browsing, and trampling animals, insects and fungi, wind, snow, and fire.

The book is certain to be widely called for and is an excellent introduction to forest life for school reading. The physiology and anatomy are very elementary, but as accurate as such general statements can be. The numerous figures and plates which are almost all half tones from photographs, constitute an attractive feature: so much overdone, however, that half of them might be spared, as far as the text is concerned.—C. R. B.

THE LAST of the *Contributions* from the United States National Herbarium (**5**: 145–259. *pls. 18–64.* 1899) is one of the most notable of the series. It contains the results of the most recent studies upon the Mexican and Central American flora by Dr. J. N. Rose, whose untiring labor both in the field and in the herbarium has resulted in large accessions to our knowledge of Mexican and Central American plants. The *Contribution* consists of two distinct parts, viz., taxonomic studies, illustrated by text cuts and ten plates; and notes on useful plants of Mexico, illustrated by thirty-seven plates of unusual excellence.

The taxonomic studies are as follows: a rearrangement of the suborder Agaveæ, in which eight genera are recognized, *Pseudobravoa* being new; synopsis of the North American species of Nissolia, including twelve species, five of them new; notes on Rutaceæ, with two new species of Xanthoxylum; notes on Turneraceæ, with a new species of Turnera; notes on Mexican species of Clitoria, with descriptions of two new species; notes on Malvaceæ and Bombaceæ, with descriptions of ten new species; notes on Passiflora, with one new species; synopsis of the North American species of Waltheria, including ten species, three of them new; notes on some Mexican species of Thalictrum, six of them being new; Cedrela, or Spanish cedar, with descriptions of two new species; notes on new or rare Leguminoseæ, fourteen new species being described; descriptions of twelve miscellaneous new species.

[2] PINCHOT, GIFFORD: A primer of forestry. Part I. The forest. 12mo., pp. 88, *pl. 47, figs. 83.* Washington: Dept. of Agric. 1899.

In addition to these Mexican studies a new genus of Commelinaceæ, *Treleasea*, is established with three species, to include certain Texan and Mexican forms heretofore referred to Tradescantia; and three new species of Tradescantia from the United States are described. A new genus of Umbelliferæ from Mt. Ranier, Washington, *Hesperogenia* by name, is described by Coulter and Rose; and Mr. L. F. Henderson describes a new Aster and a new Angelica from Idaho.

The part devoted to a description of the useful plants of Mexico is based upon the personal observations of Dr. Rose during a visit of four months in the summer of 1897. It is full of interesting information and photographic illustrations, and is very suggestive of lines of economic investigation.—J. M. C.

NOTES FOR STUDENTS

M. J. GOLDBERG's experiments lead him to the conclusion that during the germination of wheat in darkness proteid substances are produced in the embryo in considerable quantity,[3] although Godlewski in 1897 thought this to be impossible.[4]—C. R. B.

COPELAND AND KAHLENBERG, by a series of carefully conducted experiments show that the injury to plants from solutions of pure metals (Nageli's oligodynamic effect) is due to the toxicity of the compounds (salts) which the dissolved metals form and not to any peculiar or toxic action of the elemental metal.[5]—C. R. B.

M. W. PALLADINE has determined that alternations of temperature accelerate the respiration of severed tips of etiolated shoots of *Vicia Faba* cultivated in 10 per cent. cane sugar. The increased energy of respiration does not depend on the quantity of active nitrogenous foods, but the real cause is not yet determined.[6]—C. R. B.

DR J. W. HARSHBERGER has observed a distinct thermotropic curvature of leaf blade and petiole in *Rhododendron maximum* L.[7] In cold the blades are revolute and the petiole arcuate downwards. On bringing a branch into a warm room erection and flattening were complete within five minutes. The curvatures in a reverse direction are slower. Turgor variations are the cause.—C. R. B.

ITEMS OF TAXONOMIC INTEREST are as follows: GERRITT S. MILLER (Proc. Biol. Soc. Wash. **13**: 79–90. 1899) has discussed the species of Apocynum

[3] Rev. gen. de Bot. **11**: 337–340. 1899.

[4] Anzeiger Akad. Wiss. Krakau, March 1897, *fide* Goldberg.

[5] Trans. Wis. Acad. of Sci. **12**: 454–474. 1899.

[6] Revue gen. de Bot. **11**: 241–257. 1899.

[7] Proc. Phila. Acad. Sci. **1899**: 219–224. *fig. 3*.

in the District of Columbia, recognizing seven, three of them being described as new, two having been recently described by Professor E. C. Greene, and the remaining two being the well-known species of Linnaeus.— WILLIAM PALMER (Proc. Biol. Soc. Wash. **13**: 61–70. 1899) has published a list of the ferns of the Dismal Swamp, Virginia, sixteen in number, one of them being described as a new variety.— J. N. ROSE (11th Ann. Rep. Mo. Bot. Gard. 1–5. 1899) has described a new species of Agave and critical notes on other species, accompanied by four plates.— E. P. BICKNELL in his further studies of Sisyrinchium (Bull. Torr. Bot. Club **26**: 335-349, 445–457, 496–499. 1899) has added eleven new species to the already long list of forms.— AVEN NELSON in continuing his publication of new plants from Wyoming (*ibid.* 350–358, 480–487) describes twenty-three new species, one of which represents a new genus, *Nacrea*, related to Anaphalis.— C. L. POLLARD (*ibid.* 365–372) has revised the genus Achillea in North America, recognizing ten species, three of which are new.— K. M. WIEGAND (*ibid.* 399–422) presents ten species of Bidens found in the United States and Canada, describing one new species and five new varieties.— ANNA M. VAIL in continuing her studies of Asclepiadaceæ (*ibid.* 423–431) discusses the types of Gonolobus and describes three new species of Vincetoxicum.— P. A. RYDBERG (*ibid.* 541–546) has described twelve miscellaneous new species from the western United States. —A. A. HELLER in continuing the publication of his new and interesting plants from western North America (*ibid.* 547–552) describes ten new species, four of which are species of Mertensia.— J. M. C.

SOME CURIOUS experiments by A. Pagnoul[8] on transpiration are reported in the *Experiment Station Record* **11**: 118. 1899. They are difficult to explain without more light than the brief summary gives; but to call attention to them we reproduce the abstract. "Experiments are reported in which fescue grass was grown from March 30 to June 21 under almost identical conditions, the only difference being that one pot was filled with a poor clay soil without fertilizer, and the other with a rich calcareous soil to which dried blood and nitrate of potash were added. The same degree of saturation of soil was constantly maintained. The grass was cut May 2, 27, and June 21, weighed and analyzed. The results obtained are tabulated. It appears that during the first period, 33 days, the plants in the poor soil transpired 1190 ^{gm} of water per gram of dry weight; as compared with a transpiration of 555 ^{gm} in the rich soil. In the second period the figures were 1053 and 581 ^{gm} of water per gram of dry weight, and for the last period 1084 and 585 ^{gm}, respectively. The nitrogen content of the product of each pot was determined, and it was found that for each gram of nitrogen in the product of the poor soil 46 ^{kg} of water was transpired, while in the rich soil 1 ^{gm} of nitrogen was found for each kilogram of water given off.

[8] Bul. Sta. Agron. Pas de Calais **1898**: 10–15. *fig.* 1.

NEWS.

PROFESSOR DR. FRANZ VON HÖHNEL has left Vienna on a journey to Brazil.

SYNTHETIC cassia oil, introduced by Schimmel & Co. of Leipzig, is gradually displacing the ordinary cassia oil of commerce.

A PLANT of *Cypripedium insigne giganteum* from the collection of the late Major Mason, recently sold in London by auction, brought £147, and other varieties from £30 to £76. The total amount realized at the sale was over £3000.

THE HERBARIUM of the Geological Survey of Canada has recently been enriched by Professor Macoun's collections from Sable Island, by Mr. A. P. Low's from northern Labrador and the coasts and islands of Hudson's bay; by Mr. J. B. Tyrrell's from the Yukon district; and by Mr. N. B. Sanson's from the vicinity of Banff.

A COURSE for teachers, consisting of studies in ecology and plant physiology, with excursions and practice, was conducted during the autumn by Professor C. Stuart Gager, of the New York State Normal College at Albany. The syllabus shows a refreshing appreciation of the modern aspect of botany, "considering plants from a dynamic rather than a static point of view."

THE ACREAGE in peppermint in Wayne county, New York, one of the chief centers of the crop, has fallen from 3340 acres in 1890 to 300 in 1899, on account of low prices of the oil and the better returns to farmers from growing sugar beets. In Michigan the exports of peppermint oil rose from 80,225 lbs. in 1894 to 162,492 lbs. in 1897, but are now falling, being 145,375 lbs. in 1898.

WITH THE CLOSE of volume II Messrs. Willard N. Clute & Co. cease to publish *The Plant World*. It will hereafter be published by The Plant World Company, 321 4½ street, Washington, D. C. The first number of volume III will be that for January 1900, thus leaving a gap from October to December 1899 inclusive. The journal still remains under the editorial direction of Dr. F. H. Knowlton. The new volume will be more fully illustrated and nearly doubled in size, but with the same subscription price. The journal has proved very useful, and we bespeak for it the fuller success it deserves.